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Analgesic and anti-inflammatory drugs for pain management in veterinary medicine

Martina Gallo

Tutor:
Prof. Silvano Carli

Coordinator:
Prof. Valentino Bontempo

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CHAPTER 1

Introduction

1. Introduction

1.1 What is pain?

Pain is defined by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Notes associated with this definition emphasise the importance of verbal self-report in human pain assessment, but the requirement of self-report is not satisfied in such individuals as small children, mentally handicapped adults or animals (Anand and Craig, 1996). Consequently, the ability of these individuals to feel pain has often been underestimated or dismissed. However, there are strong parallels in the behavioural and physiological responses of humans and animals to noxious stimulation (Dubner and Ren, 1999), suggesting that rather than dismissing animal pain on the basis of the lack of verbal self-report, a different definition is required. The following definitions, which do not rely upon self-report, have been suggested:

"Pain in animals is an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance behaviour, and may modify species specific behaviour, including social behaviour" (Zimmerman M., 1986).

"Animal pain is an aversive sensory and emotional experience representing an awareness by the animal of damage or threat to the integrity of its tissues; it changes the animal's physiology and behaviour to reduce or avoid the damage, to reduce the likelihood of recurrence and to promote recovery; non-functional pain occurs when the intensity or duration of the experience is not appropriate for the damage sustained (especially if none exists) and when physiological and behavioural responses are unsuccessful in alleviating it" (Molony and Kent, 1997).

Owing to the complexity of the matter and the impossibility of to shortly discuss about, in the present thesis will be reported only general concepts useful to understand the basis of the studies carried out.

After a rapid introduction on pain physiology, a concise review of the methods used to evaluate pain in animals and the concepts that are on the basis of the analgesic treatments will be reported.

1.2 Physiology of pain

It is important to remember that pain that occurs after most type of noxious stimulation is usually protective and quite distinct from the pain resulting from damage to tissues or nerves. This first type of pain is called "physiologic" pain and plays an adaptive role as normal defence mechanisms, warning of contact with potentially damaging environmental insult and initiating behavioural and reflex avoidance strategies. It is characterised by an high stimulus threshold, is well localised and transient. This protective mechanism is facilitated by highly specialised network of nociceptors and primary sensory neurons that encode the intensity, duration and quality of noxious stimuli and their location (Lamont et al., 2000).

In general, the physiologic component of pain is termed *nociception*, which consists in processes of transduction, transmission and modulation of neural signals generated in response to an external noxious stimulus. It is a process that results in the conscious perception of pain when carried to completion. In its simple form, the pathway can be considered as a three neuron chain, with the first order neuron originating in the periphery and projecting to the spinal cord, the second order neuron ascending the spinal cord and the third order neuron projecting to the cerebral cortex (Figure 1). On a more complex level, the pathway involves many lines of communication with other sensory neurons and descending inhibitory neurons from the midbrain that modulate afferent transmission of painful stimuli.

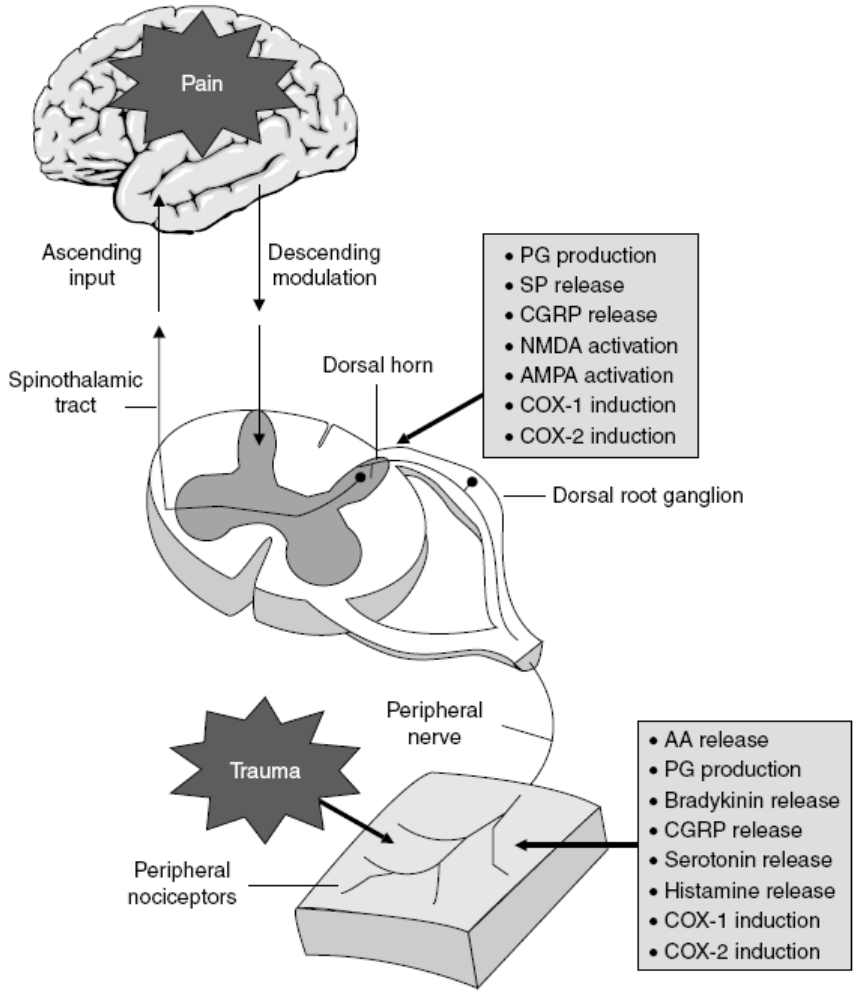


Figure 1. A simplified representation of nociceptive processing as a three-neuron chain. (Ochroch. et al., 2003)

The first process of nociception involves the encoding of mechanical, chemical or thermal energy into electric impulses by specialized nerve endings termed *nociceptors,* that have the function of preserving tissue homeostasis by signalling actual or potential tissue injury. Nociceptors are free nerve endings of primary afferent neurons and have high stimulus threshold for activation.
Most nociceptors are non-selective ion channels that are gated not by voltage but by temperature, chemical ligands or mechanical forces. After their activation, the channels open and Na^+ and Ca^{2+} ions flow into the nociceptor peripheral terminal, producing an inward current that depolarizes the membrane. The presence, specificity and threshold of these nociceptor transducers constitute the first and most important filter in nociceptive processing and define the different classes of primary afferent fibres.

The Transient Receptor Potential (TRP) ion channels are the major family involved in generating thermally and chemically evoked pain. All TRP receptors are considered to be at first thermo-receptors, but they may also respond to mechanical activators. Within the TRP family there are several subfamiles, including TRPV, TRPM and TRPA. In the TRPV family, the most important member is TRPVI (also known as the vanilloid receptor-1), responds to noxious heat (>45°C) and is also capsaicin-sensitive.
Another potentially important transducer is TREK, a member of the 2P-domain K^+ channel family, that responds to mechanical and thermal stimuli.
The action potentials are conducted from the periphery to the central nervous system along the axons of primary afferent nociceptive fibres (Lamont et al. 2000).
If the depolarizing current is of sufficient magnitude, voltage-gated Na^+ channels are activated, further depolarizing the membrane and causing a burst of action potentials, that are conducted from the periphery to the central nervous system along the axons of primary afferent nociceptive fibres. Aδ and C fibres are the principal nociceptive primary afferents and their differential activity is responsible for the unique sensory qualities of fast and slow pain (Lamont, 2008).

Unique among sensory receptors, nociceptors respond to repeated activations by lowering their threshold, thus resulting in an enhanced response to subsequent stimuli. This phenomenon is called *sensitization*. The nociceptive signals generated by nociceptor activation are transmitted to the central nervous system by their associated afferent axons, which correspond to the subclasses of nociceptors outlined previously. Aδ fibres are large-diameter (1-5 μm), thinly myelinated axons and consequently conduct impulses rapidly (5-30 m/s), thereby facilitating the first pain, which is describe as a sharp, stinging or pricking sensation, signalled by the nociceptors. On the contrary, transmission in the smaller unmyelinated C fibres (0.25-1.5 μm) is much slower (0.5-2 m/s) and acts to reinforce the immediate response of the A fibres, becoming increasingly important as the duration of the stimulus persists. These fibres mediate the "second pain" (or slow pain), a more diffuse and persistent burning sensation extending beyond the termination of an acute stimulus. Both the fibres (Aδ and C) are located throughout the skin, peritoneum, pleura, periosteum, subchondral bone, joint capsules, blood vessels, muscles, tendons, fascia and viscera, although their distribution density varies depending on the species and anatomic location (Lamont et al., 2000).

Cell bodies of both types of afferent nociceptive nerve fibres are contained in the dorsal root ganglia and extend axons to synapse with dorsal

horn neurons within the grey matter of the spinal cord. The majority of Aδ fibres terminates in the most superficial layer, lamina I (also called marginal zone), while some fibres projects more deeply to lamina V. Most C fibres are also destined for the superficial dorsal horn, with the focus in lamina II (the substantia gelatinosa). It is in the dorsal horn that initial integration and modulation of nociceptive input occur.
Primary afferent axons may form connections with three types of dorsal horn neurons:

1) interneurons, divided into excitatory and inhibitory subtypes, which serve as relays and partecipate in local processing;
2) propriospinal neurons, which extend over multiple spinal segments and are involved in segmental reflex activity and interactions among stimuli from different loci;
3) projection neurons, which participate in rostral transmission by extending axons beyond the spinal cord to terminate in sopraspinal centers such as midbrain and the cortex.

All three components are essential for the processing of nociceptive information, which facilitates the generation of an organized and appropriated response to pain.
Projection neurons have been sub-classified into three groups:

- 3a) Nociceptive-specific (NS) neurons are concentrated in lamina I and are excited solely by noxious mechanical or thermal stimuli, from both Aδ and C fibres.
- 3b) Wide dynamic range (WDR) neurons, prevalent in lamina V, receive innocuous input from low-threshold mechanoreceptors as well as nociceptive information. They respond in a graded manner over a larger receptive field than do the NS neurons and often receive convergent deep and visceral inputs and they are able to generate painful sensation after their activation.
- 3c) Complex neurons, located in lamina VII. It is believed that this cells function in the integration of somatic and visceral afferent activity (Jessel et *al.,* 1991).

The communication of nociceptive information between various neurons occurs via chemical signalling mediated by excitatory and inhibitory amino acids and neuropeptides which are produced, stored and released in the terminals of afferent nerve fibres and dorsal horn neurons.

The dorsal horn nociceptive input is conveyed to sopraspinal centers by projection neurons extending through one of several ascending pathways. The spino-thalamic tract (STT) is the most prominent nociceptive pathway in the

spinal cord. It originated from the axons of NS and WDR neurons in laminae I, V, VI and VII, which cross the midline and run in the anterolateral white matter, ultimately terminating in the thalamus. One group of STT axons projects into the lateral thalamic nuclei and transmits information from smaller and more discrete receptive fields in the periphery. Axons projecting to the medial thalamic nuclei reflect input from larger and more diverse receptive fields and are implicated in the affective-motivational dimension of pain. Comparative anatomic data demonstrated that there are species differences in the ascending fibre densities of the lateral and medial projection of the STT, assuming that, compared with primate, domestic animals have less refined stimulus characterisation and localization capabilities.
Axons of nociceptive neurons located more deeply in laminae VII and VIII form the spinoreticular tract, which ascends bilaterally in the anterolateral quadrant of the spinal cord white matter (Jessel et al., 1991).
Nociceptive neurons originating in laminae I and V project in the spinomesencephalic tract to the mesencephalic reticular formation, the lateral part of the periaqueductal grey region (PAG) and several other midbrain sites. Lesser contributions to nociceptive transmission are made from neurons located in laminae III and IV of the dorsal horn, which project axons through the spinocervical tract and the post-synaptic dorsal column pathway, which both relay impulses indirectly to the thalamus through the lateral cervical nucleus and the dorsal column nuclei, respectively. Finally, a direct projection transmitting primarily nociceptive information from the dorsal horn to the hypothalamus has been recently discovered; this is the spino-thalamic tract which provides an alternative route of activating the motivational component of pain and initiating neuroendocrine and autonomic response (Jessel et al., 1991; Cross, 1994).

The brainstem structures (medulla, pons, midbrain) supply to nociceptive function through their contributions to the reticular system and the PAG. The reticular formation is a core of neurons sending collaterals to the spinal cord, to the other reticular neurons, to various sensory and motor nuclei of the brainstem, to the diencephalon and to the cerebral cortex. Reticular neurons can mediate autonomic, motor or sensory function and although there are circumscribed areas of specialized functions within the formation, the interaction between such foci is substantial and provides the basis for unified activity of the reticular core. Ascending reticular neurons mediate the affective and motivational aspects of pain through their projections to the medial thalamus and limbic system.
The PAG of the midbrain is a major locus of integration for homeostatic control. Although noted for its importance in the descending modulation of nociceptive information, it also extends ascending projections to the thalamus

and hypothalamus, thereby providing an indirect alternative pathway for nociceptive sensory activity to reach diencephalic structures (Craig and Dostrovsky, 1997).

The thalamus serves as the relay point for sensory information directed to cerebral cortex and it is composed of numerous complexes and nuclei, several of which play key roles in nociception.

Limbic structure mediates aversive drive and thus influences the motivational component of pain and determines purposeful behaviour.

Impulse transmission to the cerebral cortex is believed to play a vital role in integrating pain perception. Although the functional and structural species differences occurring at this level are undoubtedly that the cortex is able to modulate both the cognitive and aversive affective aspects of pain sensation and to mediate increasingly complex behaviour patterns.

The most important anatomic area contributing to the endogenous analgesia system is the mesencephalic PAG. The PAG is a cell-rich region surrounding the cerebral aqueduct and it is considered to be a caudal extension of the limbic system into the midbrain. The PAG receives descending inputs from the cortex, amygdala and hypothalamus; it is modified by ascending projection from the medulla, reticular formation and spinal cord. The PAG is also involved in ascending transmission via rostral connections to thalamic, hypothalamic and limbic structures and caudal efferents project to the rostral ventromedial medulla (RVM). The antinociceptive effects observed by direct stimulation of PAG neuronal cell bodies are thought to be mediated largely by opioid activation of PAG outflow, likely operating through a GABA-containing interneuron. The dense concentration of opioid peptides and receptors found throughout the PAG underscores its importance as a substrate for opioid antinociception.

The descending nociceptive inhibition arising from PAG activation is mediated through a relay in the RVM, facilitating projection caudally to the level of the dorsal horn. Several distinct RVM nuclei are implicated in antinociception and all receive input from the PAG, send fibres to the spinal cord and contribute to endogenous opioid analgesia.

The final site involved in the descending modulation of nociceptive information is the level of the spinal cord. Dense concentration of GABA, glycine, serotonin, norepinephrine and the endogenous opioid peptides (enkephalins, endorphins, dynorphins) have been identified in dorsal horn neurons and all produce inhibitory effects on nociceptive transmission. Specifically, the spinal opioid system sets descending control mechanism by acting presynaptically (by blocking the substance P) as well as postsinaptically (Lamont et *al.*, 2000).

1.3 Nervous system plasticity

Hypersensibility is an important aspect of acute and chronic pathological pain. This phenomenon is the result of an alteration of the nervous system function, with dynamic changes at the periferical level as the reduction of the nociceptor threshold at the site of injury and at central level with an increasing in responsiveness of spinal neurons to sensory input.
Under normal physiologic situations, mechanical, thermal and chemical stimuli activate high threshold nociceptors associated with Aδ and C fibres to signal a noxious insult. In the clinical setting, however, even relatively benign noxious stimuli are associated with a degree of tissue inflammation, that initiated a cascade of sensitizing cellular and subcellular events (releasing of chemical mediators: substance P, neurokinin A, etc.) that have direct effects on the excitability of sensory and sympathetic fibres. The releasing of these mediators promotes vasodilatation with extravasation of plasma proteins and the recruitment of inflammatory cells. Mast cells, macrophages, lymphocytes and platelets with inflammatory mediators (hydrogen ions, norepinephrine, bradykinin, histamine, potassium ions, cytokines, serotonin, nerve growth factor, nitric oxide and products from the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism) act synergistically generating what is often referred as "*sensiting soup*", that lowers the response threshold for Aδ and C fibres activation (Dray, 1995; Fonda, 2009). Although changes in the afferent transduction threshold characterizing peripheral sensitization are responsible for the zone of primary hyperalgesia surrounding the site of tissue injury, they can not explain all the behavioural aspects of pain hypersensitivity seen in the clinical setting. Furthermore, the identification of a subpopulation of afferent nerve terminals called "silent" nociceptors has also contributed to understand the phenomenon of peripheral sensitization (see below).

In addition to primary hyperalgesia associated with damaged tissue, clinical or pathologic pain also invokes a heightened sensitivity in neighbouring areas not subjected to injuries (called the zone of *secondary hyperalgesia*) as well of responsiveness to normally innocuous mechanical stimuli (allodynia). Clinical hypersensitivity is the consequence of dynamic changes in dorsal horn neuron excitability. The first stage is related to the duration of the slow synaptic action potentials generated by Aδ and C fibres that have an impact on a dorsal horn neurons. These synaptic potentials may last up to 20 seconds and this results in a summation of potentials during low-frequency repeated nociceptor inputs, creating a progressively increasing and long-lasting depolarization in dorsal horn neurons. Just a few-seconds of C-fibre input can generate several minutes of post-synaptic depolarisation. This phenomenon, called "wind-up" of spinal

neurons, is mediated by N-methyl-D-aspartate (NMDA) receptors, that after activation of protein kinase C, caused the modification of NMDA channel and increase its sensitivity to glutamate. Wind-up thus contributes to the overall state of increased membrane excitability in dorsal horn neurons commonly referred to as a central sensitisation, although the two terms are not synonymous.
Central sensitisation is visible at the cellular level as a change in receptive field properties with a reduction in threshold, an increase in responsiveness, spatial extent and recruitment of novel inputs. Under normal circumstances Aβ fibres, that are large myelinated primary sensory neurons associated with highly specialized low-threshold peripheral mechanoceptors, are responsible for generating innocuous sensations; after their activation, they elicit sensations of pressure, flutter or vibration depending on the rate of adaptation of the fibre, but they never elicit pain even when high-frequency stimuli are applied. Once the dorsal horn has been sensitized by nociceptive input, however, activation of Aβ fibre mechanoceptors by previously non painful tactile stimuli actually contributes to the pain response ("silent nociceptors"). The secondary hyperalgesia and mechanical allodynia manifested clinically can be explained in term of misinterpretation of normal inputs that are not part of the physiologic pain system and would never normally generated pain but arise as a direct consequence of central sensitization. Thus, the pathophysiology of post-injury pain hypersensitivity involves dynamic changes occurring in the periphery, which enable low-intensity stimuli to produce pain by activating sensitized Aδ and C fibres, while input in low-threshold Aβ sensory fibres generates pain as a result of altered central processing in the dorsal horn of the spinal cord (Lamont et al., 2000).

1.4 Type of pain: pathologic pain

In most situations the noxious stimulus is not transient and it is associated to a tissue inflammation and a nerve injury. In this situation dynamic changes in the physiologic events are evident in both peripheral and central nervous systems. In this case pain is termed pathologic because tissue damage has already occurred, but it is also possible to call this type of pain as clinical pain, due to the discomfort and the abnormal sensitivity that characterized the patient's clinical symptomatology.
Pathologic pain may arise from injury to a variety of tissue types and it is often classified into inflammatory pain (involving somatic and visceral structures) or neuropathic pain (involving lesions of the nervous system). Furthermore, it is

possible to characterize clinical pain from a temporal perspective and make the distinction between recently occurring (acute) and long-lasting pain (chronic).

1.4.1 Inflammatory pain

It is easy to evaluate the protective and adaptive function of physiologic pain as it relates to cutaneous noxious input; however, it is less clear in the contest of deep visceral pain. The skin is subject to a constant external perturbation and nociceptive processing is vital in initiating necessary behavioural avoidance strategies. Viscera are rarely exposed to external insults but are more commonly the targets of disease processes and the protective function of pain response in this situation is not so obvious.
The sensitivity of visceral tissue to traditional types of mechanicals, thermal or chemical stimuli differs profoundly. Viscera seem more sensitive to distension of hollow muscular-walled organs (including the gastrointestinal tract, the urinary tract and the gallbladder), ischemia (myocardium) and inflammation (cystitis or pancreatitis). Visceral pain also differs from somatic pain with regard to localization. Visceral pain is perceived as being extensive and diffuse and is often associated with a sense of nausea and malaise. Referred pain, whereby the pain response is localized to distant structure, is another hallmark of visceral pain. The mechanism of this phenomenon remains a matter of considerable debate. Finally, although cutaneous hypersensitivity (primary and secondary hyperalgesia, allodynia) has been well characterized and repeatedly documented, few reports of similar changes occurring in viscera are available, although it does seem that inflammatory states in particular may predispose to visceral hypersensitivity (Fonda, 2009).

1.4.2 Neuropathic pain

Neuropathic pain is produced as a consequence of damage to the nervous system. Like inflammatory pain states, neuropathic pain is characterized by altered sensory processing of stimuli and results in several distinct and unique manifestations of hypersensitivity. Multiple mechanisms are still unknown, but two general categories of pathologic changes seem to contribute to neuropathic pain: abnormal peripheral input and abnormal central processing.
The first mechanism may arise from an acute injury discharge in axotomized afferent fibres. This situation persists for a period of 10 or more seconds and the collective effects generate a massive and aberrant input to the central nervous system. In addition to producing intense pain this input seems to generate long-lasting NMDA receptor mediate wind-up in dorsal horn neurons. Several days after this injury discharge, a second form of abnormal peripheral input develops,

with ectopic activity originating from injured axons, the proximal axonal stump (neuroma) and cell bodies in the dorsal root ganglion (Devor, 1991). This ectopic discharge is chronic and may reflect the development of abnormal sensitivity to mechanical, thermal or chemical stimuli.
The phenomenon of central sensitization also contributes to the persistence and hypersensitivity associated with neuropathic pain. Afferent fibre input may arise from chronic ectopic discharge in sensory neurons, as previously described, or it may be driven from the sympathetic neurons exciting C fibres, that have developed an adrenergic sensitivity secondary to axotomy (Campbell et al., 1992).
An additional form of altered central processing is observed in neuropathic pain states and involves structural reorganization in the cell bodies of injured axons in the dorsal root ganglion. Studies have demonstrated that axotomized Aβ fibres sprout from their normal site of termination in the deeper laminae of the dorsal horn into the superficial laminae I and II, which are normally occupied by Aδ and C fibres. Nerve injury also stimulates sympathetic fibres to sprout around large dorsal route ganglion cells, providing another mechanism whereby post-axotomy sympathetic activity may activate nociceptive afferents.
Abnormal central processing as a result of a persistent state of central sensitization or dorsal horn structural reorganization may provide an unifying explanation for neuropathic pain mediated by sympathetic and Aβ fibres (Lamont et al., 2000).

1.4.3 Acute pain

This type of pain occurs from soft tissue trauma or inflammation, e.g. after surgical operation. In this case, acute pain has a biologically adaptive function by facilitating tissue repair and healing. This is achieved by hypersensitizing the injured area (primary hyperalgesia) as well as the surrounding tissues (secondary hyperalgesia) to all type of stimuli such that contact with any external stimulus is avoided and the reparative process can proceed.
After surgery, there are many variable reactions at the different levels of the nervous system.
Among systemic effects, it is possible to identify:

- Supraspinal or segmental: consists in an increased sympathetic tone accompanied by peripherical vasoconstriction, increased cardiac output, myocardial work and skeletal muscle tone and decreased gastrointestinal and urinary tone
- Endocrine: release of corticotropin, cortisol, antidiuretic hormone, catecholamines, renin, angiotensin II, aldosterone, glucagone and

interleukin 1, with concomitant decreased in insulin and testosterone secretion.

- Stimulation of brainstem centers: increasing of respiratory rate, bronchospasm.
- Anxiety and fear that enhance the sympatetic response, underlined previously, and contribute to increase blood viscosity, to prolong clotting time, fibrinolysis and platelet aggregation. These effects derived from a nociceptive stimulation at the diencephalic and cortical level (Lamont et al., 2000).

These effects represent the typical response to an acute pain. The magnitude and duration are related with the degree of tissue damage (days or months).

1.4.4 Chronic pain

Chronic pain persists beyond the expected time frame for a given disease or injury and has been arbitrarily defined as having a duration greater than 3 to 6 months. In recognition of the multifactorial nature of this type of pain, the International Association for the Study of Pain has incorporated more than 200 clinical syndromes in their classification of chronic pain, with cancer pain, osteoarthritic and postamputation phantom limb pain among the most relevant to the veterinary practitioner. In all cases, chronic pain is maladaptative and offers no useful biologic function or survival advantage. Therefore, chronic pain implies more than just duration, it is a debilitating affliction that has a significant impact on a patient quality of life and it is often characterized by a scarce response to conventional analgesic treatment.
In the future, a further understanding of the neuromechanisms of this type of pain will carry to establish new strategies to control chronic pain (Lamont et al., 2000).

1.5 Pain evaluation

Veterinary practitioners have recently increased their attention about pain management and improvement of health status and welfare of their patients. Untreated pain decreases quality of life, prolongs recovery from surgery or illness and, in some cases, could induce anatomical damages that lead to persistent pain, hyperalgesia or allodynia.
The increasing attention in animal welfare leads to better quality and longer life of our domestic animals but also a greater risk of development of chronic and

painful pathologies (mainly musculoskeletal and oncologic) (AAHA/AAFP 2007).

Recently, there has been an increased focus on determining and measuring species-specific pain behaviours, which should improve recognition and treatment of pain in animals. Nevertheless, the assessment of pain in animals remains a subjective and inaccurate undertaking. Numerous factors complicate the evaluation of pain in animals.

Physiologic parameters (e.g., changes in heart rate, respiratory rate, arterial blood pressure, pupil dilation) may be used to assess responses to an acute noxious (painful) stimulus, particularly during anaesthesia, and to assess pain in some clinical situations (e.g., horses with acute colic pain).

There are two different types of evaluation: objective and subjective. The first includes the evaluation of some physiologic parameters (heart rate, respiratory rate, blood pressure), the amount of stress-indicator substances (cortisol, glicemya) and the response to analgesic therapy; the subjective evaluation is based on pain score scales.
Actually, the clinical evaluation of pain in animals is mainly based on subjective methods, where a personal interpretations of animals behaviour may lead to an under- or over-estimation of pain. On the other hand, the exclusive consideration of objective parameters, although useful in pain evaluation in awareness patient or after general anaesthesia, are not predictable of pain because often there are no differences in patients undergoing surgery and in control group, and they are always altered also in stressed patients (Hellyer, 2002).
During intra-operatory period, the evaluation of End-tidal percentage and of the minimal alveolar concentration (MAC) of the anaesthetic gas, used to induce and maintain anaesthesia, are considered two valid tools in assessing pain level in this period.
The first parameter represents the % of an anaesthetic gas at the end of expiration. It is characteristic for each patient.
While MAC % is the minimal alveolar concentration of the anaesthetic gas, able to maintain in anaesthetic conditions the 50% of subjects undergone a supramaximal stimulus. This value is standard for each gas (e.g. the MAC% of Isoflurane is 1.3), but it may change after coadministration of analgesic or sedative drugs, lowing its value.

1.6 Pain score scales

Pain assessment in animals is a current important issue and has been investigated by many Authors. The pain evaluation is well described and validate in humans patients. Therefore, these methods have been currently adapted to animals.

Development and use of species-specific pain scoring system would greatly facilitate evaluation of pain in veterinary patients.
Any pain scale should consider the following characteristics: species, breed, environment and rearing conditions, age, gender, cause of pain (e.g., trauma, surgery, pathology), body region affected (e.g., abdominal pain, musculoskeletal pain), character of pain state (e.g., acute, chronic) and pain intensity. Any pain scale or methodology employed to assess pain should be able to recognize individual sensitivities. All pain scales give a subjective evaluation of pain degree, that is made from an human observer.
It is obvious that its judgment will be conditioned by its previous painful experiences. Moreover, the observer should be careful not to be influenced by the tendency of anthropomorphizing animal pain.
When assessing pain in animals, it is important to observe the behaviour and its response to analgesic therapy over the time. The assessment must be appropriate for the age of animal because younger animals are much less tolerant of pain. However, this should not be interpreted as exaggerated puppy or kitten behaviour, resulting in an inadequate use of analgesics. Based on character, some breeds of dogs (small and toys breed) show more emphasis in pain manifestations; otherwise pain may be incorrectly treated in some of the largest worker breeds that have a reputation of being "stoic".

1.6.1 Descriptive scale

According to this scale, pain assessment should be classified in few descriptive categories (no more than five), i.e. absence, medium, moderate, serious and intolerable pain. It resulted being easy to apply, but of weak sensibility. Descriptive scales have been initially used for humans since 1975, but they resulted of difficult management in animals (Holton et al., 2001).

1.6.2 Visual analogue scale (VAS)

In this case, pain assessment must be into a value between 0 to 100, placed on an horizontal line, with a length of 100 mm, where the value 0 is no pain and unbearable pain is the value 100. When the total score obtained is more

than 60, a rescue analgesia is request. In Veterinary medicine, VAS is used for the evaluation of acute pain (Holton et al., 1998), post-operative pain in dog (Firth and Haldane, 1999) and cat (Cambridge et al., 2000).
To avoid possible mistakes, it is necessary that the observer is well trained to recognize animal behaviour during pain status and to discriminate species differences.

1.6.3 Numerical score scale

Pain assessment is obtained by the sum of the scores of different descriptive categories (e.g. vocalization, unprovoked behaviour, palpation, etc.) attributed by a trained observer. In general, the use of numerical scales lead to a more complete evaluation of pain degree than others previously described, because of their simplicity in score assigning; however, it has not yet exceeded the personal interpretation of the observer.
This scale is the most used in Veterinary medicine, with adaptation to the different species. In 1998 in the Colorado State University a scale was adopted with eight variables (attention, movement, attitude of the eyeball, vocalization, unprovoked behaviour, response to palpation, heart and respiratory rate) for a total score of 24 points.
Other similar scales were studied to satisfy the necessity to assess pain level in our domestic animals.
The short-form Glasgow Composite Measure Pain Scale (CMPS) is a behaviour-based composite scale to assess acute pain in dogs. It takes the form of a structured questionnaire completed by an observer following a standard protocol which includes assessment of spontaneous and evoked behaviours, interactions with the animal and clinical observations. The questionnaire consists of seven behavioural categories: posture, activity, vocalisation, attention to wound or painful area, demeanour, mobility, and response to touch. In each category are grouped a number of words or expressions from which the observer chooses that one in each category which best describes the dog's behaviour. A list of specific definitions for each item helps to ensure consistent use between observers. Ranked scores are summed, the maximum pain score is 24, or 20 if mobility is impossible to assess, in case of heavy surgery (e.g. limbs, spinal or pelvic fractures).

Smith et *al.* (2004) used a score scale to evaluate the analgesic activity of lidocaine, after intra-ocular surgery in dogs. This scale consisted of six aspects to evaluated in each dog: Comfort, Movement, Appearance, Behaviour, Interactive behaviour and Vocalization. Every parameters were assessed by a trained observer unaware of the treatment and a total score between 0 to 24 were assigned. Any dog that had a total subjective pain score ≥ 9 or a score ≥ 3 in

each category received rescue analgesia (morphine: 1 mg/kg, IM) and was excluded from further data recording (Smith et *al.,*. 2004).

1.7 Pre-emptive and multimodal analgesia

Today, complex surgery can be carried out on extremely fragile and elderly patients; the anaesthesiologist has taken on a greater role in managing the patient during the peri-operative period (i.e. pre-operative, intra-operative and post-operative periods). As part of this evolution, control of postoperative pain takes on a fundamental role, which goes beyond assuring proper analgesia, with the aim to ensure comfort and quality of life. Pre-emptive analgesia seems to constitute one of the most innovative and promising strategies for better pain control throughout the peri-operative period.
An accurate definition of pre-emptive analgesia remains under debate; however, the physio-pathological aspects on which pre-emptive analgesia are based are well defined. Pre-emptive analgesia refers to pharmacological intervention initiated prior to a painful stimulus in order to inhibit nociceptive mechanisms before they are triggered. This block must be maintained throughout the whole surgical period as well as during the postoperative phase.
Pre-emptive analgesia has three objectives: to reduce pain resulting from the activation of inflammatory mechanisms generated by surgical incision; to hinder the pain memory response of the central nervous system and to ensure a good control of postoperative pain in order to avoid the development of chronic pain. To determine the efficacy of pre-emptive analgesia *versus* traditional methods, the drug must be administered to one group of patients prior to surgery and its efficacy compared with a second group of patients who received the drug after surgery. Indeed, the key-point on which the pre-emptive analgesia method is based is the moment in which any given drug is administered and not the choice of the drug itself. Therefore, if a drug is administered before surgery it is more effective, from the point of view of the analgesia than a drug administered in the post-surgical phase (Grape and Tramèr, 2007).

The preoperative administration of non steroidal anti-inflammatory drugs (NSAIDs) is very common in veterinary medicine, although still under discussion due to its possible effects on clotting function and renal perfusion in the event of hypotension occurring during anaesthesia. NSAIDs administration before surgery is more effective, if compared to postoperative administration, in blocking the production of autacoids involved in the process of peripheral sensitization (Hanlon et al., 1996; Boccara et al., 2005).

Blocking peripheral sensitization decreases sensory and nociceptive inputs towards the spinal cord, contributing to controlling spinal cord neuroplasticity. In cases where soft tissue damage and a strong inflammatory response are expected, pre-emptive NSAID administration should be considered, after careful evaluation of the possible side-effects.
Clinical studies have shown the efficacy of pre-emptive NSAIDs in animals, although it is not always possible to administer the drug before the insult, as in the case of trauma or pre-existing conditions (Welsh et *al.*, 1997).
Surgical stimulation is so intense that it is almost impossible to effectively block the nociceptive inputs using a single drug, even if it belongs to the analgesic-opioid class, unless dangerously high doses are administered. The most effective approach to control surgical pain consists of using an appropriate loco-regional technique, which achieves complete block of sensory inputs, and therefore has a pre-emptive effect in the strict sense of the term, as shown by several studies in humans. Loco-regional anaesthesia, however, is not always an exploitable choice, and its diffusion in veterinary medicine is not as wide as in human anaesthesia. The major drawback of relying on loco-regional techniques as sole analgesic is the possibility of inadequate efficacy, caused by choice of the wrong technique, by an incorrect execution or by the necessity of extending the surgery outside the anaesthetized area (Corletto , 2007).

Multimodal (or balanced) analgesia consists of using several drugs, with different mechanism of action, interfering with perception, transmission, and modulation of pain. Although most anaesthetists will agree on the fact that multimodal analgesia allows a more effective treatment of pain, they will not necessarily agree on its practical application.

The basis of balanced analgesia is, as already mentioned, the use of combinations of drugs affecting nociception (in the anaesthetized patient), pain (in the conscious patient) and neuroplasticity. The major advantage of this approach, immediately perceived, is the reduction of the dose of the drugs used, decreasing the occurrence of side-effects. In theory the different mechanisms of action of the drugs ensure more effective control of pain at different levels.
The use of combinations of several drugs introduces a significant variable, most of the times unpredictable, which is the effect of one drug on the effect site concentration of the other drugs. This unpredictable interaction may result, in the best case scenario, in unexpected effects and, in the worst case, may be a source of morbidity.
When two or more drugs are used together, in the event of an anaesthetic accident, it may be very difficult, if not impossible, to correctly address the cause of the problem. Drawing a line between an extremely conscientious approach (polypharmacology) and an effective one (multimodal analgesia) may be very

difficult, and in some instances impossible, especially considering that the best index of outcome (patient welfare) is very difficult to assess objectively in veterinary medicine.

Since every analgesic intervention has to benefit the patient, in the planning the analgesic protocol it is necessary to consider not only the surgery but also the patient. The temperament of the patient should affect the choice of the drugs to treat pain in the peri-operative period in veterinary medicine as it does in human beings. The exaggerated sympathetic response, typical of a stressed and anxious patient, counteracts the effect of most of the analgesic agents used and should be properly addressed.

An uncontrolled stress response may also affect neuroendocrine homeostasis and aggravate peri-operative complications.

Another factor deeply affecting peri-operative pain is the surgeon. Any experienced anaesthetist will confirm that the same surgery performed on the same patient by two different surgeons may result in a completely different analgesic requirement.

Accordingly, the analgesic protocol should be adjusted not only to the surgery and the patient, but also to the surgeon. The surgeon affects the duration of the procedure and the extent of tissue damage. Inexperienced surgeons will require a longer time to perform the surgery, and cause more tissue damage, affecting peri-operative analgesic demand.

The most common approach to multimodal analgesia in pre-anaesthetic medication in veterinary patients is to include a tranquillizer/sedative, an opioid, and a non-steroidal anti-inflammatory drug (NSAID). Whether to include or not a sedative depends on the patient's temperament, and on local operating procedures. Omission of a sedative may be desirable in healthy and calm patients undergoing day surgery and requiring a quick discharge. On the other hand, when dealing with aggressive or potentially aggressive patients, deep sedation may be required in order to ensure the safety of personnel. If a sedative has to be included in the premedication protocol, an $\alpha 2$ agonist is probably the best choice, as careful dose titration leads to the desired sedation (from light to deep) with minimal cardiovascular and respiratory side effects, modulates the spinal processing of pain via descending inhibition, and finally has agonistic effects with opioids (Corletto , 2007).

1.8 Pharmacologic considerations for management of pain

It is possible to obtain analgesia with pharmacologic treatment operating in different levels of pain nociception, so that the importance to know the

different mechanisms and ways through pain is perceived, transmitted and modulated.

Actually, there are few pharmacologic interventions that acting on transduction. One exception is capsaicin, an algogenic substance present in hot pepper. It binds to TRPV1 receptors and activates them, which initiates a pain response. A prolonged topical application of capsaicin has been showed to desensitize TRPV1 receptor and to produce analgesia.
The local anaesthetics (e.g. lidocaine and bupivacaine) are classic Na^+ channel blockers; their main effect is to inhibit nerve impulse conduction along Aδ and C fibres, thereby blocking the nociceptive signals to the central nervous system.
The α2-agonists, alone or in combination with local anaesthetics, may inhibit impulse conduction when applied perineurally.
The principal analgesic drugs that act in the dorsal horn are the opioids, the α2-agonists and NSAIDs.
Dense population of opioid receptors exist in the dorsal horn and activation of these receptors may have pre- and post-synaptic effects. At the pre-synaptic level, decreased Ca^{2+} influx reduces the release of excitatory transmitter substances, such as substance P, from primary afferents, with consequent inhibition of nociceptive transmission. Postsinaptically, enhanced K^+ efflux causes hyperpolarization of projection neurons, which also inhibits ascending nociceptive pathways. Owing to α2-adrenoceptors belong to the same superfamily as do opioid receptors, the α2-agonists have a similar mechanism of analgesic action within the dorsal horn.
The NSAIDs are widely used in veterinary medicine to manage various types of pain. Although their peripheral antiprostaglandin effects make them an obvious choice to minimize development of peripheral sensitization of nociceptors, they also inhibit cyclooxygenase (COX) within the spinal cord dorsal horn and thus are considered to have central-acting analgesic effects.
The opioids are the class of drugs that play the most significant role in descending nociceptive modulatory pathways through their actions at multiple levels, including PAG and the dorsal horn. Despite the potential for adverse side effects associated with their administration, opioids remain among the most powerful and efficacious analgesics available.
Because of the importance of NMDA receptors in central sensitization, their inhibition is a common analgesic strategy. Ketamine acting on NMDA receptors is able to reduce the early phase of central sensitization and the resultant hypersensitivity to pain. Due to the wide diffusion of NMDA receptors throughout the brain, psychomimetic side effects arise, which limit its clinical use. Amantadine is another drug with anti-NMDA effects: it cause the closing of

these channels rather than the block of current flow through open channels and this activity assures a more safe profile of the drug (Lamont, 2008).

1.9 Objectives

Due to the recently increased interest in the animals welfare, the pain therapy has been deeply investigated and newer drugs should be available, mainly for pets treatments. However, the number of drugs currently licensed for the use in veterinary medicine is still low, so that the off-label use of analgesic is a quite common practice. It should be considered that the dose extrapolation between humans and animals or even among different animal species is rather incorrect, since many species-specific differences has been demonstrated. Therefore, dosing extrapolation might induce an increase of adverse effects or even the lack of the intended effect.

According to the concepts of the pre-emptive analgesia, the current trend is to administer analgesic treatment before the application of the pain stimuli. Among the main interest of the veterinary practitioner, the management of peri-operative pain is very important and represents one of the most frequent case where practitioners are called to intervene.

In this study two drugs were selected to be evaluated under pharmacokinetic profile and their analgesic efficacy during the peri-operative period in dogs, cats and horses.
Tramadol has most recently been used in veterinary medicine as effective analgesic agent with a reliable metabolism, useful in those patients that do not tolerate the most commonly used drugs to control surgical and chronic pain (e.g. NSAIDs). Due to the great differences in the metabolic behaviour of the drug in the animal species, pharmacokinetic parameters associated to analgesic efficacy must be established for each species. Pharmacokinetic studies of tramadol and its metabolites indicate interspecies differences in drug metabolism supporting the fact that pharmacokinetic studies are necessary in order to determine dosage regimens to achieve targeted plasma concentrations which are associated with analgesia in humans (Scott and Perry, 2000; KuKanich and Papich, 2004; De Sousa et *al.*, 2008).
Due to the favourable and potent anti-inflammatory and analgesic activities, Ketorolac (NSAID) could represent a useful tool to control acute pain also in animals, such as in the post-operative period. Although ketorolac is not currently approved for use in veterinary medicine, its efficacy of this drug was evaluated by Mathews et *al.* (1996) and they showed that ketorolac was equivalent or more

effective than the narcotics butorphanol and oxymorphone and the flunixin meglumine in relieving pain after arthrotomy o laparotomy in dogs (Mathews et *al.*, 1996).

1.10 Tramadol

Tramadol (4-phenyl-piperidine; TRA) is a centrally acting drug structurally related to codeine. It was first synthesized in 1962 and has been available for pain treatment in Germany since 1977. It acts as a μ receptors agonist and it also inhibits serotonin and norepinephrine re-uptake. TRA is indicated in treatment of moderate to severe pain and it has also effects against allodynia and neuropathic pain. Despite its analogy with codeine and its μ receptors activity, considering the low incidence of collateral effects typical of this category (respiratory depression, constipation and drug dependence), TRA is not usually considered as a true opioid (Grond and Sablotzki, 2004). In Italy, the registration of TRA in veterinary medicine was made for dog (Altadol®, Formevet SpA), in two pharmaceutical forms: solution for intravenous or intramuscular injection and soluble tablets for oral administration.

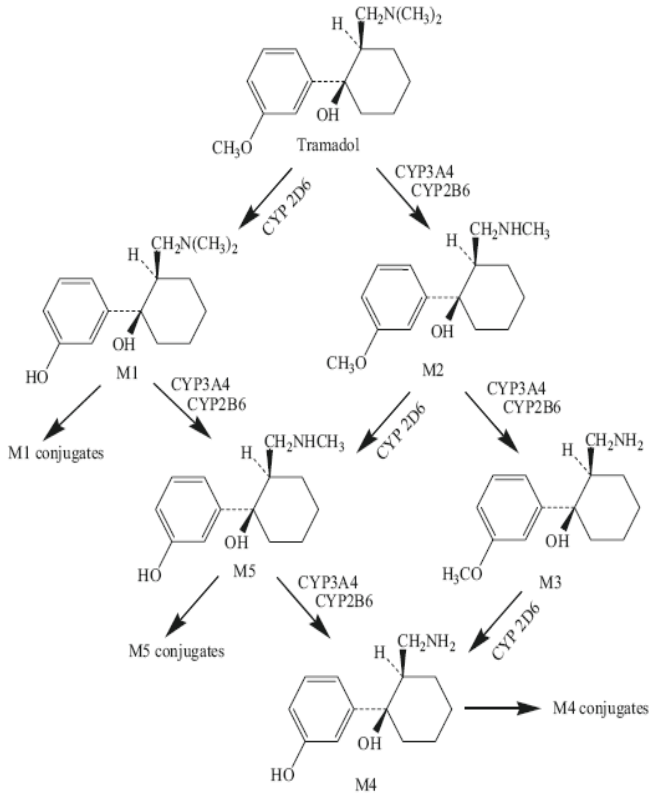
TRA is both a weak opioid agonist with selectivity for the μ-receptor and a weak inhibitor of the reuptake of noradrenaline (norepinephrine) and serotonin (5-hydroxytryptamine; 5-HT). This dual mechanism of action may be attributed to the two enantiomers of racemic TRA. The (+)-enantiomer has a higher affinity for the μ-receptor and is a more effective inhibitor of 5-HT reuptake, whereas the (–)-enantiomer is a more effective inhibitor of noradrenaline reuptake and increases its release by autoreceptor activation. Since endogenous norepinephrine and serotonin are involved in central pain modulation, these properties may thus enhance the analgesic effects of TRA produced by its opioid binding activity (Scott and Perry, 2000).
This binary mechanism of action of TRA may explain the reduced potential for abuse as well as less significant respiratory depression and other adverse effects typically attributed to traditional opioids. This may also explain because TRA has been reported to be effective in the control of chronic pain conditions that show reduced sensitivity to opioids (McMillan et al., 2008).

Metabolism of TRA occurs in the liver through two main metabolic pathways to produce active and inactive metabolites.
The primary metabolites of Phase I, the O-desmethyl-tramadol hydrochloride (termed M1) and N-desmethyl-tramadol (M2), may be further metabolised to

three additional secondary metabolites, namely N-Ndidesmethyl- tramadol (M3), N-N-O-tridesmethyl-tramadol (M4), and N-O-didesmethyltramadol (M5).
The M1 metabolite has 2 to 4 times the analgesic potency of the parent compound and 4 to 200 times greater affinity for the μ-receptor (Lewis and Han, 1996); it also exists as a racemic mixture: the (+) enantiomer having affinity for the μ-receptor and the (-) enantiomer having affinity for adrenergic receptors. Studies in rats showed that administration of the (-) enantiomer of M1 metabolite alone resulted in no antinociceptive activity; however, it was capable of potentiating the antinociceptive effects elicited by (+) M1 enantiomer (Garrido et *al*., 2000).

In humans, the metabolic fate of TRA is modulated by cytochrome P450 (CYP). The isozyme CYP2D6 catalyzes the reaction of O-demethylation forming M1. N-Demethylation reaction is conducted by CYP2B6 and CYP3A4. These isozymes form the inactive metabolite M2 from TRA and M5 from M1 (Subrahmanyam et al., 2001). The metabolism process continue with the Phase 2 reactions that produce sulfates and glucoronides conjugates of the Phase 1 metabolites (Figure 2).
The enzyme metabolism of TRA in animals is still unknown and needs to be better investigated.
It will be of interest to determine if there are differences in enzyme metabolism between canine breeds as occurs in human populations; this variety may comport implications in administration of TRA as analgesic agent (McMillan et al., 2008; Cox et al., 2010).
In humans, hepatic impairment will result in decreased metabolism of the parent compound and the active metabolite, resulting in a greater area under the plasma concentration curve (AUC) and prolongation of elimination half-life ($T_{½β}$). In humans, elimination half-life increases with renal insufficiency as tramadol and its metabolites are primarily excreted via the kidneys (90%) with the remaining 10% being excreted via feces (McMillan et *al.* 2008). Approximately 10 to 30% of the parent drug was excreted unmetabolized in the urine (Scott and Perry, 2000).

Figure 2. Main metabolic pathways of Tramadol in human (Lintz et *al.,* 1981).



1.10.1 Clinical use of tramadol in animals

The TRA analgesic activity in dogs during post-surgical period was tested in Italy, alone or combined with other analgesic drugs. Study concerning analgesia after *routine* surgery showed that TRA efficacy was obtained in 53% of patients (dosage: 4 mg/kg, IM). To treat chronic pain, TRA in combination with NSAIDs had more analgesic activity (90%) than by itself (60%). A comparison between TRA and Flunixin meglumine in surgical pain control showed better awakening and more sedation grade with the first compound. Recently, TRA is used also as additional drug in treatment of chronic, neuropathic, osteo-articular inflammatory and neoplastic pain (Fonda, 2009).
Currently, the use of TRA in cats is quite limited due to the low ability in glucuronidase activity in these species.
Pain control in post-operative period was evaluated in cats undergone surgery. They were anaesthetized with Medetomidine and Ketamine, then TRA at the dose of 2 mg/kg, IM was administered. The analgesic effect obtained in these patients was 100% (Fonda, 2009).

In cats, a comparison of analgesic activity between TRA and Butorphanol, administered at the dosage of 2 mg/kg IV after surgery, showed a substantial equivalence in relieving pain (Ravasio et *al.*, 2007).
Although TRA is not widely investigated in horses yet, it is considered by practitioners as a good option from practitioners to control moderate to severe pain. In last years, TRA was used to control colic and surgical pain in horses, with increasing frequency (Bhuvanakumar, 2005; Cassu et *al.,* 2006).
TRA analgesic activity was also investigated in other species as reptile, laboratory animals (rats, mice, rabbits, hamsters), birds and fishes. In reptiles and birds, that possess more δ and k receptors respectively, TRA shows similar NSAIDs analgesic activity and limited adverse effects (Johnston, 2005).

1.11 NSAIDs in veterinary medicine

NSAIDs are one of the most widely used drug classes in human and veterinary medicine. The general health of the patient influences the decision to use NSAIDs: cats and dogs are more susceptible than humans to the adverse effects and the reported safety of an analgesic drug in a human patient should not be assumed to be the same in a veterinary patient.

The analgesic properties of NSAIDs can be attributed to their inhibition of COX and the subsequent decrease in prostaglandines (PG) both in periphery and central nervous system. COX is the enzyme that catalyzes the transformation of arachidonic acid to unstable endoperoxide intermediates (PGG_2 and PGH_2).
The discovery of two isoforms of COX more than ten years ago motivated efforts to identify selective inhibitors of both enzymes. The properties of a third recently identified COX isoform (COX-3) are not well-known nowadays but probably it is an isoform of COX-1 (Chandrasekharan et *al.,* 2002).
COX-1 is constitutively expressed in both the peripheral and central nervous system and its expression can be induced by a number of factors including many of the mediators of pain and inflammation. COX-2 is ubiquitous in the central nervous system, but it is not present in periphery except in the kidneys and vas deferens. COX-2 becomes a major enzyme for PG production in the periphery only after induction. The COX-2 isoform is up-regulated by bacterial lipopolysaccarides, cytokines, growth factors, tumour promoters and multiple factors released during cell damage and death, including the mediators of pain and inflammation.

In the periphery, initial PG release is due to COX-1 as it takes 2-8 hours for maximal COX-2 messenger RNA to be expressed. However, in the central nervous system, COX-2 is constitutively expressed and also can be induced.
Inhibition of peripheral PG production is important for decreasing nociceptive transmission to the central nervous system. The major contribution of NSAIDs to decreasing hyperalgesia is through inhibition of spinal PG production (PGE_2). The action of PGE_2 in the dorsal root ganglia leads to increases in Ca^{2+} conductance and decreases in rectifying potassium (K^+) current, leading to a net enhancement of excitability. Consequently, less nociceptive input is required from the periphery to project nociceptive input to the brain. Overall, inhibition of spinal PGE_2 production by NSAIDs that inhibits COX-2 appears to be the primary mechanism for preventing hyperalgesia in animal models (Ochroch et *al.*, 2003; Yask et *al.* 2001).
The ability of NSAIDs to inhibit the formation of PG is related to their capacity to reach the central nervous system. It depends from the distribution and final concentration of drug within the central nervous system. The rate concentration of the compound from blood to CNS is determined by plasma concentration, protein binding, physical-chemical properties, cerebral blood flow and blood-brain barrier permeability (Lorenzo and Spector, 1976).
In conclusion, NSAIDs that inhibit COX-1 and COX-2 may have a more immediate impact on pain by hindering PG production in the periphery and CSN.

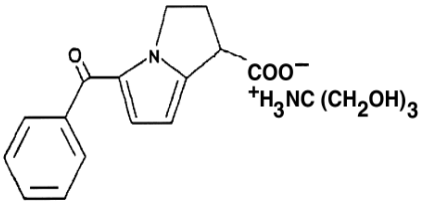
At the present, the administration of NSAIDs should only be considered in well-hydrated normotensive patients, with normal renal and hepatic functions, with no haemostatic abnormalities, no evidence or concern for gastric ulceration and not receiving corticosteroids.

In an attempt to treat pain before it occurs, the practice of pre-emptive analgesia is encouraged. Various studies using pre-operative administration of NSAIDs in veterinary patients did not show adverse reactions ensuring an excellent analgesia (Grisnaux et *al.*, 1999).
Although current evidence in young healthy dogs suggests that pre-operative administration of NSAIDs may seems to be beneficial, this type of administration should be used with caution, as no prove of its higher efficacy and safety has been reported in clinical trials. Whether intra-operative fluid support and attention to systemic blood pressure are indicated in patients that received NSAIDs pre-operatively is another area requiring investigation (Mathews, 2000).

1.12 Ketorolac

Ketorolac (KET), an heteroaryl acetic acid derivative, is a NSAID approved for use in humans that possesses potent anti-inflammatory, analgesic and antipyretic activities (Figure 3) (Sinha et *al.*, 2009). It is commercially available as a mixture of racemate of tromethamine salt, where the S(-)-enantiomer is more biologically active than R(+)-enantiomer (Gillis and Brogden, 1997). Its properties depend on prostaglandin synthetase inhibitory activity and its effectiveness is comparable to morphine, but without troublesome side effects as constipation and respiratory depression (Anthony and Jasinski, 2002).

Figure 3. Chemical structure of ketorolac tromethamine. (Pasloske et *al.,* 1999)



In humans, KET is extensively metabolized primarily by coniugation with glucuronid acid; *para*-hydroxylation represents a minor pathway (≈ 12%). The metabolites have no significant analgesic activity.
Urinary excretion was the major route of elimination, with approximately 90% of the dose in humans recovered in urine and the remainder in faeces (Jung et *al.*, 1989).
As with most NSAIDs, KET is highly bound to the plasma proteins (>99%) and, as expected, the apparent volume of distribution is limited. In mice the highest concentration were found in kidney, liver and lungs and the lowest in muscle, gonads and spleen (Brocks et *al.,* 1992).
Literature data shows that KET kinetic parameters are modified in the elderly and in patients with renal dysfunctions: in both the elimination rate of KET was reduced, probably due to a lower clearance (Buckley and Brogden, 1990).

In humans KET is used to control the symptomatic relief of moderate to severe postoperative pain, including that associated with abdominal, gynecologic, oral, orthopedic or urologic surgery (Sinha et *al*, 2009). The KET parenteral administration is advisable for a maximum of 5 days to limit its side effects, such as gastrointestinal, haematological, renal and neurological reactions (Martindale, 2005).

The analgesic and anti-inflammatory activities of KET should be investigated. Consequently, several different COX-independent activities of KET were examined in an effort to explain its marked analgesic efficacy in comparison to other NSAIDs. These mechanisms included facilitation of extracellular calcium entry , indirect activation of k opioid receptors and modulation of nitric oxide (NO) synthase. Currently, none of these activities have been conclusively shown to account for the analgesic efficacy or potency of KET *in vivo*.

Although several studies have shown that KET does not cross the blood-brain barrier in both rats and humans after peripheral administration, evidence that KET acts in the central as well as the peripheral nervous system to produce analgesia has been accumulated. For example, after intrathecal administration, KET blocks pain states associated with central sensitization: formalin-induced hyperalgesia in rats and thermal hyperalgesia in a neuropathic rat model (Jett et *al*., 1999).

Some authors suggest the existence of a further mechanism of action of KET at the level of NMDA receptors, but it has not been demonstrated yet (Sotgiu et *al*., 1998).

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CHAPTER 2

Pharmacokinetics and efficacy of intravenous and extradural tramadol in dogs

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The recognition of animal pain as a medical entity and therefore an ethical problem has received increasing scientific attention over the previous decades and has resulted in the promulgation of animal welfare laws.

The neuraxial administration (spinal and extradural) of local anaesthetics or analgesics is used effectively to manage perioperative pain. Extradural (ED) injection at the lumbosacral site are particularly useful after operations involving the pelvic viscera and, or limbs, e.g., in dogs, tibial plateau levelling osteotomy (TPLO). In human beings, ED tramadol has been used in both adults and children, but with contrasting results (Baraka et al., 1993; Delilkan and Vijayan, 1993; Prosser et al., 1997; Siddik-Sayyid et al., 1999; Yaddanapudi et al., 2000; Ozcengiz et al., 2001; Güneş et al., 2004; Demiraran et al., 2005; Prakash et al., 2006).

Some studies have been published on the pharmacokinetics and analgesic properties of tramadol in cats, horses and goats (Cagnardi et al., 2006; Zonca et al., 2006; De Sousa et al., 2008; Pypendop and Ilkiw, 2008; Shilo et al., 2008). Using dogs, Kukanich and Papich (2004) studied the pharmacokinetic properties of intravenous (IV) and oral (PO) tramadol at 4.4 mg/kg and 100 mg/animal respectively, as well as M1 (1 mg/kg IV). Mastrocinque and Fantoni, (2003) compared tramadol (2 mg/kg) with morphine (0.2 mg/kg) after IV injection for the control of post operative (ovariohysterectomy) pain and found tramadol produced analgesia equivalent to morphine. Preliminary data on tramadol pharmacokinetic behaviour in dogs confirmed the extradural route as an effective route for providing analgesia for surgical techniques (Vettorato et al., 2006).

This study investigated the pharmacokinetic profile of tramadol and its M1 metabolite in dogs after the IV and ED injection of 2 mg/kg, as well as the effects of administration route on post-operative analgesia in dogs undergoing TPLO.

Materials and methods

Animals

The study involved 10 client-owned dogs, 5 male and 5 female, of various breeds (group ED: 1 Golden Retriever, 1 Deutsch-Kurzhaar, 1 Dogue de Bordeaux, 1 Pitbull and 2 Cross breed; group IV: 4 Cross breed) and ages (2-9 years old) weighing between 10 and 43 kg, admitted for surgery at the Veterinary School of Padua for the surgical repair of ruptured cranial cruciate ligaments using the TPLO technique (Slocum and Slocum, 1993).

All animals were judged to be healthy (ASA I-II) on the basis of the physical and haematological examination, and were enrolled in the study after the written consent of the owners following the Italian Regulation by D.L. 116/1992.

Anaesthesia

In all animals, food, but not water, was withheld from the evening before the surgery. Pre-anaesthetic medication in all cases was intramuscular (IM) acepromazine (0.02 mg/kg, Prequillan, Fatro) and pethidine (4.0 mg/kg, Petidina Cloridrato, Monico). Twenty-five min later anaesthesia was induced with propofol (Rapinovet, Schering-Plough) injected over 30 seconds to effect (2.0 - 4.0 mg/kg). The animal's trachea was then intubated and anaesthesia maintained with isoflurane (Isoba, Schering-Plough) using 100% oxygen delivered by a re-breathing system. All the surgeries were performed by the same surgeon.

Tramadol administration

In all animals the area included between the L7-S1 vertebra was surgically prepared and then each dog was randomly assigned to receive tramadol (2 mg/kg, Altadol, Formevet) by either ED or IV injection. The randomization was performed by extraction from envelop. The ED injections were performed at the lumbosacral space as described elsewhere (Skarda, 1996). In brief a 20 SWG Thuoy needle (Perican Paed, B-Braun) was advanced into the extradural space and its accurate position confirmed by the absence of blood and, or cerebrospinal fluid at the hub, and the perception of minimal resistance to drug injection using a low resistance syringe (Perifix, B-Braun). In each dog receiving ED tramadol, the drug was diluted with sterile saline solution to produce a total volume of 1 mL/10 kg body weight.

All injections were made by the same anaesthetist who subsequently abstained from effect evaluation. Conversely, the evaluator was unaware of the route of drug administered. Injection of tramadol was considered as time zero (t = 0), for both the pharmacokinetic and efficacy study.

Pharmacokinetic study

Serum samples

Blood samples were collected into non-heparinized tubes before (time 0) and 5, 10, 20, 30 min, and 1, 2, 4 and 8 h after administration. Serum samples were prepared by centrifugation (1500 g, 10 min at room temperature) and stored at –80°C pending assay.

Reagents and SPE extraction

Tramadol hydrochloride and M1 were supplied from Formevet S.p.A. Isolute SPE C2 columns were purchased from International Sorbent Technology LTD. All reagents and solvents were purchased from J.T. Baker.

Tramadol and M1 were extracted from 500 μL serum aliquots diluted with 500 μL of 0.05 M sodium hydrogen phosphate dodecahydrate solution and briefly vortexed. Samples were purified by solid phase extraction with Isolute SPE C2 (100 mg/mL) cartridges activated with 2 mL of methanol and 2 mL of 0.05 M sodium solution. Columns were washed with 2 mL of 0.05 M sodium solution and compounds were eluted with 1 mL of methanol. The eluate was evaporated to dryness under nitrogen flow at 45°C and then reconstituted with 100 μL of mobile phase.

HPLC analysis

The plasma concentrations of tramadol and M1 were determined by HPLC equipped with a binary pump (Perkin Elmer, Series 200), an auto sampler (Perkin Elmer, Series 200), a Peltier column oven (Perkin Elmer, Series 200) set at 20°C and a fluorescence detector (Perkin Elmer, LC240) with excitation and emission wavelength of 200 nm and 301 nm, respectively. Tramadol and M1 were separated by an ODS Hypersil C18 250 X 4.6 mm 5 μm column with Hypersil 5 μm 4.6 mm pre-column purchased from Supelco. The mobile phase consisted of a solution of 15 mM sodium hydrogen phosphate dodecahydrate with 45 mM triethylamine (J.T. Baker) pH 3 and acetonitrile (82:18, v:v). Flow was 1.0 mL/min and 50 μL was the injection volume.

Spiked solutions for the calibration curve were prepared diluting the original stock solution of tramadol and M1 (1 mg/mL) to reach concentrations ranging from 0.05 μg/mL to 10 μg/mL in dog blank serum.

Serum protein binding

Dog serum protein binding was determined in vitro for tramadol and M1 in the concentration range 0.5 - 2.0 μg/mL and 0.5 -1.0 μg/mL, respectively. The free compounds were separated by ultrafiltration (Villa et al., 1994; Villa et al., 1997) using a disposable device (Amicon, Millipore) and analyzed by HPLC as described above.

Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 5.0.1 software (Pharsight Corporation) which

allows compartmental and non-compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value.

Serum tramadol concentrations after IV injection were fitted to standard bi-exponential equations (Gibaldi and Perrier, 1982) describing two-compartment models with elimination from the central compartment. Parameters estimated by the model were used to calculate the other pharmacokinetic parameters for each dog, the volume of distribution (V_c) in the central compartment as follows:

$$V_c = Dose/C_0$$

Tramadol pharmacokinetics after ED administration and changes of M1 concentration in plasma after both ED and IV injection were determined with standard non-compartmental equation.

Mean residence time (MRT), mean absorption time (MAT), body clearance (Cl_B) and volume of distribution at steady state (V_{dss}) were determined using the following equations:

$$MRT = AUMC/AUC$$
$$MAT = MRT_{ED}\text{-}MRT_{IV}$$
$$Cl_B = Dose/AUC$$
$$V_{dss} = Cl * MRT$$

The bioavailability (F%) of tramadol after ED administration was calculated as the ratio of the area under serum concentration-time curves ($AUC_{(0\text{-}\infty)}$) after ED and IV administration:

$$F\% = (AUC_{ED}/AUC_{IV}) * 100$$

Statistical analysis

Pharmacokinetic parameters are reported as means with standard deviation (SD), harmonic means were calculated for half-lives, and pseudostandard deviations (SE) were calculated using a jack-knife technique (Lam et al., 1985). InStat 3.0 (GraphPad Software) was used to perform the analyses.

The principal tramadol and M1 kinetic parameters were compared after IV and ED administration using unpaired *t*-tests with Welch corrections (variances unequal). Differences with $P<0.05$ were considered significant.

Efficacy evaluations

Clinical observations

Heart rate (HR), the electrocardiogram (Lead II), respiratory rate (*f*r), end-tidal carbon dioxide tension ($PE'CO_2$), end-tidal isoflurane tension (PE'Iso), non-invasively measured mean arterial blood pressure (MAP), haemoglobin saturation determined by pulse oximetry (SpO_2) and oesophageal temperature were recorded every 5 min (Cardiocap II - Capnomac Ultima, Datex-Ohmeda).

Postoperative pain assessment

Postoperative pain was assessed by the same observer, using the Short Form of the Glasgow Composite Pain Scale (SF-GCPS) (Reid, 2007) at 0, 30, 60, 90, 120, 240, 360 and 480 min after tracheal extubation. When scores exceeded 5/20 or 6/24 (taken to indicate moderate – severe pain) then rescue analgesia (methadone 0.2 mg/kg) was administered by IM injection. The observer was not aware of the drug administered.

Statistical analysis of efficacy parameters

All data were analysed using InStat 3.0 (GraphPad Software) software and the results were expressed as mean ± SD.

The means of age, body weight, time between tramadol administration and the beginning of surgery and the duration of surgery were compared between groups using an unpaired *t*-test. The same test was also used to compare the mean of the following variables between the two groups (HR, fr, MAP, PE'CO2 and PE'Iso). $P<0.05$ was considered to indicate statistical significance. The Mann-Whitney Rank Sum Test was applied to analyse the data obtained from the SF-GCPS; $P<0.05$ was considered to indicate statistical significance

Results

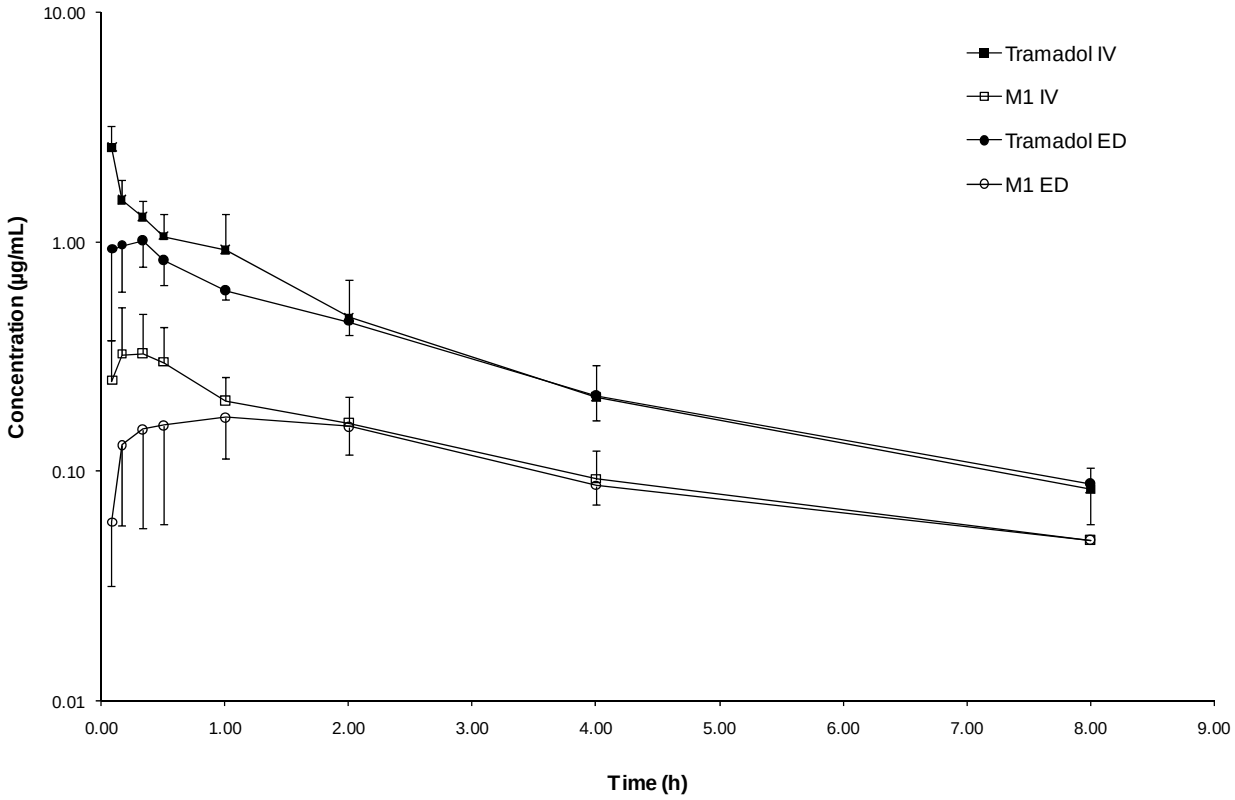
Pharmacokinetic study

The retention times for tramadol and M1 with the analytical HPLC method adopted were 11.8 and 5.2 min respectively. The HPLC method was subjected to intra-laboratory validation and found to be specific, linear (in the

range 0.05 – 10.00 μg/mL) precise and accurate, with a limit of quantification of 0.05 μg/mL for both the compounds investigated. The limit of detection (LOD) was 0.73 ng/mL for tramadol and M1.

Tramadol was administered extradurally in 6 dogs (group ED) and intravenously in other 4 dogs (group IV). Mean serum concentrations (± SD) of tramadol and M1 after IV and ED administration are shown in Figure 1. After IV administration mean tramadol concentration (±SD) in serum was 2.59 (±0.63) μg/mL at first sampling (5 min), decreased to 0.93 (±0.39) μg/mL at 1 h and reached 0.08 (±0.02) μg/mL at 8 h. M1 concentration appeared rapidly in serum, reached a plateau (0.32±0.2 μg/mL) at 10 min that was maintained for at least 2 h. The limit of quantification was observed at the last sampling point (8 h).

Figure. 1. Mean serum concentrations (μg/mL) ± SD of tramadol and M1 in dogs after IV (n. 4) and ED (n. 6) tramadol administration at the dose of 2 mg/kg.



After ED administration stable tramadol concentrations of approximately 1 μg/mL were detected from the first sampling time (5 min) till 30 min and decreased to 0.09 (±0.03) μg/mL at 8 h after injection. M1 concentrations had a similar profile to those after IV administration but with lower plateau concentrations of about 0.15 μg/mL.

The percentage of protein binding obtained was approximately 15% for tramadol and about 17% for M1. Pharmacokinetic data after IV and ED injection are compared in Table 1.

Table 1 Pharmacokinetic parameters (mean±SD) of tramadol and o-desmethyl tramadol (M1) after IV and ED administration in dogs.

Parameters (units)	Group IV (n. 4)		Group ED (n. 6)	
	tramadol	M1	tramadol	M1
$t_{1/2\lambda z}$ (h)		3.59±0.88[a]	2.66±0.50[a]	3.77±1.74[a]
AUMC (h.h.μg/mL)	13.04±11.33	6.51±2.20	10.29±3.29	9.59±9.29
$MRT_{(0-\infty)}$ (h)	2.63±1.13	5.42±2.54	3.47±0.65	7.02±4.24
MAT (h)			0.84	
$AUC_{(0-\infty)}$ (h.μg/mL)	3.56±0.67	1.24±0.16	2.92±0.36	1.19±0.43
$t_{1/2\lambda 1}$ (h)	0.10±0.16[a]			
$t_{1/2\lambda 2}$ (h)	2.24±0.87[a]			
C_0 (μg/mL)	5.48±5.92			
V_{dss} (mL/kg)	1995.89±1165.24			
V_c (mL/kg)	637.24±344.57			
Cl_B (mL/h.kg)	1748.99±1239.67			
F %			82	
C_{max} (μg/mL)		0.35±0.17	0.18±0.12	0.20±0.08
T_{max} (h)		0.29±0.16[b]	1.15±0.31	1.14±0.72[b]

[a] harmonic mean ± pseudo SE; [b] significantly different ($P<0.05$); $AUC_{(0-\infty)}$ = area under serum concentration-time curve; $t_{½\lambda 1}$ = distribution half-time; $t_{½\lambda 2}$ = elimination half-time; $t_{½\lambda z}$ = elimination half-time; C_0 = serum concentration at time 0; V_c = volume of distribution in central compartment; Cl_B = serum clearance; AUMC = area under the moment curve; $MRT_{(0-\infty)}$ = mean residence time; MAT = mean absorption time; V_{dss} = volume of distribution at steady state; F = bioavailability; T_{max} = observed time for C_{max}

Efficacy evaluation

No adverse effects were observed after IV (4 dogs) or ED (6 dogs) tramadol injection. The mean age, mean body weight, duration of surgery and the time between the tramadol administration and the beginning of the surgery are reported in Table 2. No statistical differences were recorded. Furthermore, no statistically significant differences were revealed when the means of the cardiorespiratory variables were compared between groups. The mean value (±SD) of each variable is reported in Table 3.

Table 2 Mean (±SD) of the age, body weight, surgery time and time between tramadol administration and the beginning of the surgery in the 2 groups.

	Group ED (n. 6)	**Group IV** (n. 4)
Age (year)	4.3 (± 2.3)	4.7 (± 2.9)
Body weight (kg)	33.9 (± 7.8)	22.7 (± 8.6)
TPLO time (min)	129 (± 15.1)	124 (± 16.3)
tramadol injection - start surgery (min)	20 (± 1.4)	21.3 (± 1.7)

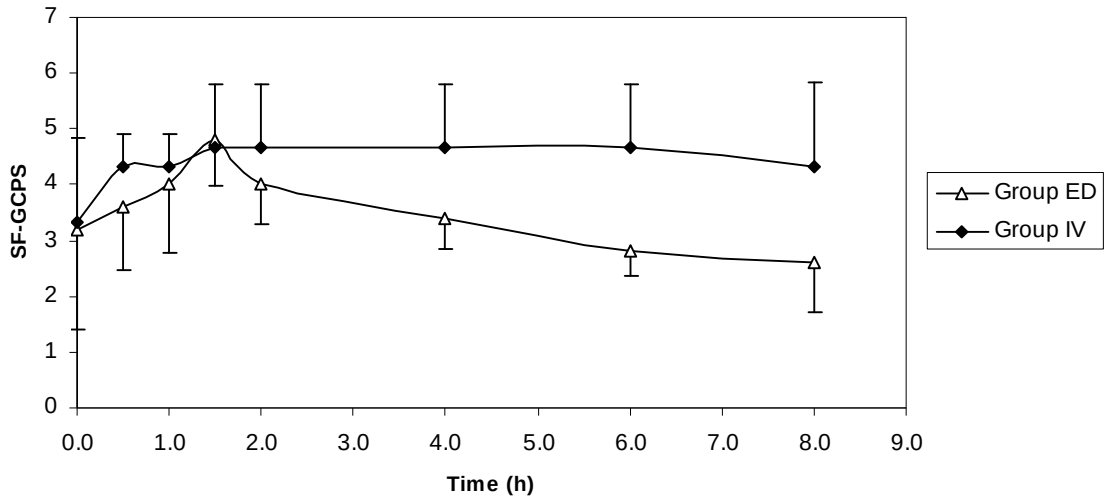
Table 3 Mean values (±SD) of perioperative cardiorespiratory variables and postoperative pain scores recorded in the two groups.

	Group ED (n. 6)	**Group IV** (n. 4)
PE'Iso (%)	1.45 (± 0.06)	1.28 (± 0.13)
PE'CO_2 (kPa [mm Hg])	6.15 (±1.10) [46.14±8.28]	5.55 (±0.30) [41.61±2.26]
heart rate (beats/min)	106.45 (± 12.35)	99.51 (± 10.57)
mean arterial pressure (mmHg)	71.91 (± 17.30)	68.74 (± 3.8)
fr (beats/min)	10.81 (± 2.99)	11.24 (± 1.32)
SF-GCPS	3.7 (± 0.3)	4.14 (± 1.34)

PE'Iso = end-tidal isoflurane tension; PE'CO_2 = end-tidal carbon dioxide tension; *fr* = respiratory rate; SF-GCPS = short form of the Glasgow Composite Pain Scale.

Statistical analysis of the "pain" data obtained using the SF-GCPS indicated that no difference was present between the two groups. The mean (±SD) of each group is reported in Table 3 and Fig. 2 shows the trends of the pain evaluation scores assessed during the 8 postoperative h.

Figure 2. Postoperative pain evaluations in the group ED (n. 6) and IV (n. 4) using the short form of the Glasgow Composite Pain Scale.



Discussion

The dose used in the current study was that recommended by the manufacturer of Altadol (Formevet), which is the veterinary medicinal product authorized in Italy for PO and IV use in dogs.

In the present study, the dogs were enrolled on the basis of surgical requirement (TPLO), thus breeds, gender, body weight and ages were different and not standardized. Therefore variability was noticed for concentrations and for mean tramadol and M1 pharmacokinetic parameters after both routes of administration. Indeed after IV injection volumes of distribution and clearance were lower and elimination half-lives were longer than those reported by Kukanich and Papich (2004).

Immediately after tramadol administration M1 attained concentrations greater than 10.0 ng/mL for the whole observation period. In humans, this value is considered the lowest concentration associated with therapeutic efficacy (Lehmann et al., 1990).

The rapid and effective production of M1 was observed after both IV and ED administration but drug concentration at the plateau were three times higher after IV administration compared to ED injection. The values of M1:tramadol AUC ratio after IV and ED administration were 0.35±0.04 and 0.48±0.16, although not statistically different, indicated a slightly higher production of M1 after ED treatment. The higher variability observed after ED could be attributed

to the absorption process dependent on the depth and location of ED injection site.

Tramadol's bioavailability was not determined exactly (there were different subjects for each administration group) but, comparing the mean AUCs the relative amount of drug exposure was high (82%) in the ED group. Furthermore, the M1 profile was similar to that after IV injection albeit with lower plasma concentrations. No significant differences were observed between routes, except for M1 T_{max}, that appeared longer after ED injection. Our results agreed with those of Murthy et al. (2000) who, investigating tramadol disposition in children, described its rapid transfer into the systemic circulation after ED administration and concluded that analgesia produced after ED injection was not attributable to systemic availability alone.

Pharmacokinetic and pharmacodynamic studies in human beings have demonstrated that the analgesic effects of tramadol results mainly from (+) M1. However, synergistic interaction between tramadol enantiomers and M1 enantiomers cannot be excluded (Poulsen et al., 1996). Several investigators have reported that in humans, the demethylation reaction to produce M1 is catalyzed by isoenzyme cytochrome P-450 2D6 (CYP2D6), an isoform which shows genetic polymorphism (Poulsen et al., 1996, Kukanic and Papich, 2004) and supports the finding of variable M1 elaboration in people. As reported by Shah et al. (2007), using bufuralol as CYP2D6 probe in dogs and female cats, the catalytic activity in these species was higher than in humans. The data obtained in our study are similar with those derived from cats by Pypendop and Ilkiw (2008) confirming the good M1 production observed in our dogs. As CYP2D6 polymorphism has been observed in rats, humans and cats it is likely that also in the present study gender, age and breed of enrolled dogs might influence M1 formation rate and tramadol disappearance in vivo.

There are a few published studies describing the analgesic properties and efficacy of tramadol in animals (Natalini and Robinson, 2000; Mastrocinque and Fantoni, 2003; Kukanic and Papich, 2004; Pypendop and Ilkiw, 2008). Natalini and Robinson (2000) evaluated the analgesic effects of ED tramadol in horses and found that 1 mg/kg increased the pain threshold to noxious electrical stimuli. Complete analgesia (avoidance threshold, >40 V) in the perineal and sacral areas was achieved after 30 min with a duration of analgesia of 4 h.

During human paediatric inguinal herniotomy, ED tramadol (2 mg/kg) produced longer postoperative analgesia and a lower requirement for rescue analgesia compared to groups receiving 1 and 1.5 mg/kg by the same route

(Prakash et al., 2006). In our study the dose of tramadol was that suggested by Prakash and colleagues.

Neither IV nor ED injection of tramadol produced any observable adverse effects on the dogs included in the current study.

According to Mastrocinque and Fantoni (2005) the analgesic effects of IV tramadol (2 mg/kg) administered are equivalent to those produced by IV morphine (0.2 mg/kg) in dogs undergoing ovariohysterectomy surgery. It is not appropriate to compare the data in the current study with this previous work because of differences in the type of surgery (ovariohysterectomy *vs* TPLO) the "type" of pain produced (visceral *vs* orthopaedic), the anaesthetic technique and pain scoring system utilized. A simple descriptive scale, associated with the measurement of plasma catecholamines, serum cortisol and glucose concentration, was used by Mastrocinque and Fantoni. However, according to multiple studies (Reese et al., 2000; Lemke et al., 2002; Grisneaux et al, 1999) visual and descriptive scale, the measurement of serum cortisol and glucose concentration can be poor predictors of postoperative analgesia in dog.

While the mean end-tidal isoflurane tension (PE'Iso) in dogs receiving IV tramadol was lower (1.28%) than that obtained in the ED group (1.45%) statistical analysis failed to identify significant differences between groups. This is in part explained by the pharmacokinetic data revealed by the current study: despite high bioavailability after ED injection, IV administration produces higher M1 concentrations (which possesses a 200 to 300 times greater affinity for μ receptors than tramadol itself). However, M1 production depends on the systemic absorption and subsequent hepatic metabolism of tramadol, which is limited after ED injection. Tramadol's analgesic effect after ED injection is the effect of tramadol alone, and receives little contribution from M1. However, common with Mastrocinque and Fantoni (2005), the mean PE'ISO (%) required to maintain anaesthesia in both IV and ED tramadol recipients was lower than typical values recommended for surgical anaesthesia, i.e., 1.2 - 1.4 X the minimum alveolar concentration which is approximately 1.6-1.9 % for isoflurane in dogs (Thurmon et al., 1999). While the contribution of propofol can be considered negligible due to its short duration of action, acepromazine, known to decrease the MAC of halothane in dogs (Heard et al., 1986), has probably influenced the sparing effect of isoflurane obtained in our study. The presence of a control group would have been helpful to better understand this effect but it would have been unethical to perform an orthopaedic surgery without using any analgesic.

The recognition and quantification of pain in animals is complicated by their inability of communicating, and also by intraspecific and individual variability in pain's manifestation. For these reasons the use of a pain scoring system is helpful to assess the efficacy of an analgesic technique. The ideal scale should be objective, reliable, repeatable and easy to use. So far, several pain scoring systems (visual analogue scale, numerical rating scale, simple descriptive scale and composite scale) were developed, utilized and correlated in dog (Conzemius et al., 1997; Holton et al. 1998; Buback et al., 1996; Firth and Haldane, 1999). Unfortunately, all of them are subjective even if they include the measurement of physiological variables (heart rate and respiratory rate). Further, their reliability and repeatability can be doubtful. The numerical pain score utilized by Reese et al. (2000) failed to detect significant differences between the placebo and the treatment group when carprofen (2 mg/kg PO every 12h) was used as the only analgesic in dogs undergoing orthopaedic procedure. However, in the latter study, the pain scores were not assigned by the same individual each time. Nevetheless, care must be taken not to confuse a lack of statistical significance and lack of efficacy when analgesic drugs are compared.

In our dogs, the postoperative pain was evaluated using the short form-Glasgow Composite Pain Scale (Reid et al., 2007) for 8 h after tracheal extubation (Fig. 2). To increase the level of objectivity and reliability all the measurements were performed by the same observer unaware of the treatment administered. The SF-GCPS did not show any significant difference between groups and no dogs required rescue analgesia at any time.

In male children, aged 1-3 years, ED tramadol (2 mg/kg) injection increased and prolonged postoperative pain relief for up to 24 h compared with IV injection (Güneş et al., 2004). No rescue analgesics were required in the ED group. On the contrary 30 out of 34 children belonging to the IV group required pethidine. In our study, despite greater antinociceptive effects of IV injection observed, long term analgesia appears to be similar after injection by either route. This may be contested on the grounds that: a) the 8 h observation period was too short (Güneş et al., 2004); b) the assessment of postoperative pain lacked objectivity; and c) there were very few animals in each group. Unfortunately, there are currently no objective pain scales suitable for use in animals. The SF-GCPS is internationally recognized while the use of the same observer, ignorant of the route of drug administration, should increase the level of objectivity (Reid et al., 2007).

Conclusions

The ED administration of tramadol (2 mg/kg) in dogs undergoing TPLO produced adequate intra- and postoperative analgesia without significant side-effects. However, the analgesia produced by ED tramadol was not superior to that obtained after IV administration. For these reasons, the ED route could not be considered as a practical alternative to IV tramadol in dogs.

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CHAPTER 3

Pharmacokinetics, intraoperative effects and postoperative analgesia of tramadol in cats

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The present study reports (a) the pharmacokinetic profile of tramadol and its M1 metabolite in cats after IV administration at 2 mg/kg prior to surgical gonadectomy, and (b) a clinical evaluation of the efficacy of tramadol as postoperative analgesic. The aim of the study was to generate data for the rational dosing of this substance in cats.

Materials and methods

Animals

The study was performed on 12 healthy domestic shorthair cats, age 0.5-1.5 years, 6 males and 6 females, weighing between 2.5 kg and 4.3 kg, undergoing gonadectomy at the Department of the Clinical Veterinary Sciences, University of Milan. All animals were judged healthy (ASA status I) on the basis of physical examination and results of routine blood tests, and were enrolled in the study after written consent from their owners, as required by Italian law (D.L. 116/1992). Subsequently, the protocol was approved by the Ethical Committee of the University of Milan.

Anaesthetic and surgical procedures

All animals received atropine sulphate (0.03 mg/kg) and acepromazine maleate (0.05 mg/kg) intramuscularly (IM), as pre-anaesthetic medications. Anaesthesia was induced with isoflurane in oxygen (100%) using an anaesthetic chamber. After intubation anaesthesia was maintained with the same gases, delivered by a non-rebreathing system (Mapleson C). Tramadol (2 mg/kg) was administered IV as a bolus over 15 seconds through a cephalic catheter (22 gauge) 5 min after intubation and 20 min prior to beginning surgery. During surgery lactated Ringer's solution was administered at 5 ml/kg/h through the same catheter. Female cats underwent ovariectomy and the males underwent orchiectomy according to standard surgical procedures. During surgery, heart rate, electrocardiogram (lead II), respiration rate, oxyhaemoglobin saturation, end tidal carbon dioxide (CO_2), mean non-invasive arterial blood pressure, and end tidal isoflurane concentration were recorded every 5 min using a UT4000F Pro monitor (Goldway Inc.).

After extubation subjective pain scores were assessed by a trained observer using a method modified after Smith et al. (2004). The method involves assessment of behavioural indicators of pain (comfort, movement, appearance, unprovoked behaviour, interactive behaviour and vocalization) assigning a score of 0 to 4 for each. Thus a score of 24 indicates maximum pain and a score of zero no pain. Pain was assessed every 30 min up to 6 h. Buprenorphine (10 μg/kg IM) was administered if the pain score was 9 or above.

Collection, purification and analysis of serum samples

For 8 animals (4 males and 4 females), venous blood samples (2 ml) were collected into non-heparinized tubes from a jugular vein catheter: before tramadol administration (time 0) and 5, 15, 30, 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 h after tramadol administration. The samples were centrifuged (1500 g, 10 min at room temperature) soon after collection and the serum stored at –80°C pending assay.

Serum samples were purified by solid phase extraction on Isolute SPE C2 (100 mg/ml) cartridges (International Sorbent Technology Ltd., UK) previously activated with 2 ml of methanol followed by 2 ml of 0.05 M sodium chloride. Five hundred μl of 0.05 M sodium hydrogen phosphate dodecahydrate solution was added to 500 μl of serum and briefly vortexed. The sample was then loaded onto the cartridge followed by washing with 2 ml of 0.05 M sodium chloride. The compounds were eluted with 1 ml of methanol. The eluate was evaporated to dryness under nitrogen at 45°C and the residue dissolved in 100 μl of mobile phase.

Residues were analysed for tramadol and M1 by HPLC. The apparatus included a binary pump, auto sampler, Peltier column oven (all Perkin Elmer Series 200, Italy) at 20°C, and a fluorescence detector (Perkin Elmer LC240, Italy) with excitation and emission wavelengths 200 nm and 301 nm, respectively. The column was an ODS Hypersil C18 250x4.6 mm 5 μm column with Hypersil 5 μm 4.6 mm pre-column (Supelco, Italy). The mobile phase was 15 mM aqueous sodium hydrogen phosphate dodecahydrate with 45 mM triethylamine pH 3 and acetonitrile (82:18, v:v). Flow rate was 1.0 ml/min and injection volume was 50 μl.

Solutions for the calibration curve were prepared diluting stock solutions of tramadol and M1 (1 mg/ml) to obtain concentrations in the range 0.05 to 10 μg/ml in blank cat serum.

HPLC retention times were 11.5 min for tramadol and 5.4 min for M1. The HPLC method was validated in our laboratory and found to be specific, linear (in the range 0.05–10 μg/ml) precise (CV 2.05–7.4 % for tramadol and 3.8–9.6 % for M1) and accurate (-13% – +0.1% for tramadol and -0.02 – +2.5 % for M1), with limit of quantification 0.05 μg/ml and limit of detection 0.0008 μg/ml for both compounds investigated. The mean recoveries for tramadol and M1 were 98.6 ± 6.86 % and 92.9 ± 4.6%.

Serum binding of tramadol and M1 in the range 0.5–1 μg/ml was determined in vitro. The serum-bound molecules were removed by ultrafiltration (Villa et al., 1994, 1997) using a disposable device (Amicon, Millipore, Italy) and free substances in the filtrate were analyzed by HPLC as described above.

Tramadol hydrochloride was kind gift from Formevet; M1 was purchased from Sigma. Other reagents and solvents were purchased from J.T. Baker (Italy).

Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 5.2.1 software (Pharsight Corporation, USA) which allows compartmental and non-compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model that best fitted the data. All data points were weighted by the inverse square of the fitted value. Serum concentrations after IV tramadol administration were fitted to a standard bi-exponential curve (Gibaldi and Perrier, 1982) describing a two-compartment model with elimination from the central compartment.

Parameters estimated from the model were used to calculate pharmacokinetic variables for each animal. The volume of distribution in the central compartment (V_c) was calculated as:

$$V_c = Dose/C_0$$

where *Dose* is dose of tramadol and C_0 is the extrapolated serum concentration of tramadol at time 0. The kinetics of M1 was determined by non-compartmental analysis. Mean residence time (MRT), body clearance (Cl_B) and volume of distribution at steady state (V_{dss}) were determined from the following equations (Gibaldi and Perrier 1982):

$$MRT = AUMC/AUC$$
$$Cl_B = Dose/AUC$$
$$V_{dss} = Cl_B * MRT$$

where *AUMC* is area under the moment curve and *AUC* is area under serum concentration-time curve.

Statistical methods

Means and standard deviations (SD) of intraoperative variables and pain scores were calculated for male and female cats separately. The *t*-test and the

Mann-Whitney rank sum test were used to estimate the significance of differences, with $P<0.05$ considered significant. The analyses were carried out with the GLM-SigmaStat 2.03 software.

Pharmacokinetic parameters were reported as means (SD); harmonic means with pseudo-standard deviations were calculated for half lives using a jack-knife technique (Lam et al., 1985). To assess sex-related differences, kinetic parameters for tramadol and M1 in male and female animals were compared by unpaired *t*-test with Welch correction (variances unequal); differences with $P<0.05$ were considered significant.

Results

Intra-anaesthetic evaluation and postoperative analgesia

Mean age, body weight, duration of surgery, selected surgical variables, and subjective pain scores are shown in Table 1.

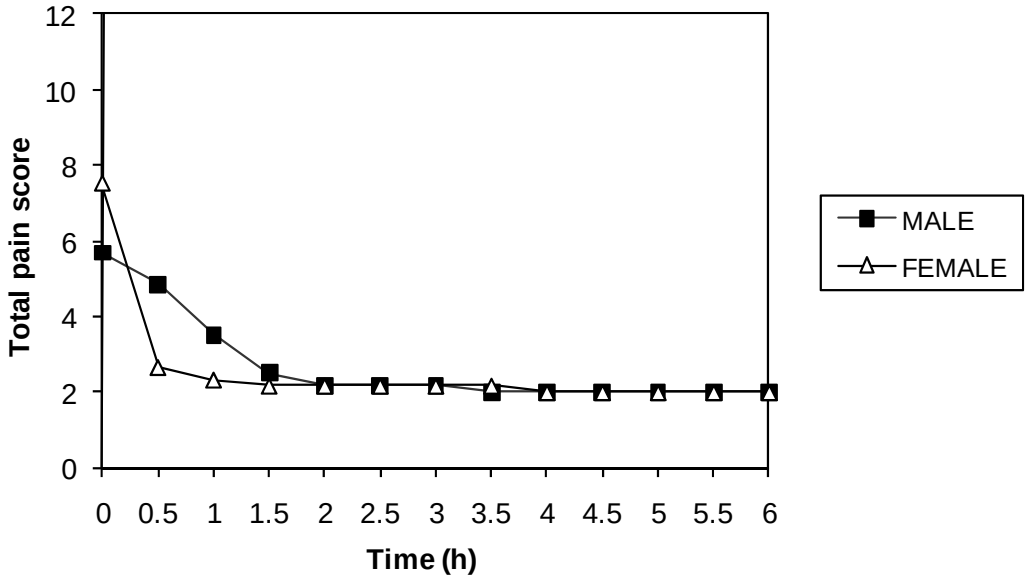
Table 1: Mean (±SD) values of general characteristics, selected surgical variables, and subjective pain score in 12 cats undergoing surgical gonadectomy

	Males (n. 6)	**Females (n. 6)**	**Males + females (n. 12)**
Age (months)	11 ± 4.5	10 ± 4.6	10 ± 4.4
Body weight (kg)	3.6 ± 0.7	2.8 ± 0.2	3.7 ± 1.1
Surgery time (min)	14.5 ± 3.7	28.5 ± 3.4	21.5 ± 8
Time from tramadol injection to start of surgery (min)	20	20	20
Heart rate (per minute)	120.3 ± 13.6	134.9 ± 16.3	128.7 ± 16.7
Respiration rate (per minute)	37.6 ± 8.1	30.6 ± 12.2	33.6 ± 11.2
End tidal CO_2 (mmHg)	41.5 ± 2.5	41.6 ± 2.3	41.5 ± 2.4
Mean non-invasive blood pressure (mmHg)	67.2 ± 4.2	66.7 ± 6.8	66.9 ± 5.4
Oxyhemoglobin saturation (%)	>98	>98	>98
End tidal isoflurane (%)	1.3 ± 0.3	1.5 ± 0.4	1.4 ± 0.4
Subjective pain score	2.7 ± 1.2	2.6 ± 1.5	2.6 ± 1.4

The mean duration of surgery was 14.5 ± 3.7 min for males and 28.5 ± 3.4 min for females. No significant differences between males and females in terms of cardiovascular and respiratory variables during the surgery were found. Although the mean isoflurane requirement in female cats (1.5 ± 0.4 %) was higher than in males (1.3 ± 0.3 %), the difference was not significant. Normocapnia (end tidal CO_2 in range 35-45 mmHg) and spontaneous ventilation were maintained in all animals throughout the procedure.

The results of the subjective pain evaluations over the 6 h after extubation are shown in Fig. 1.

Figure 1. Total pain scores in males (n = 6) and females (n = 6) assessed at various times after extubation obtained using a subjective pain scoring method modified after Smith et al. (2004).

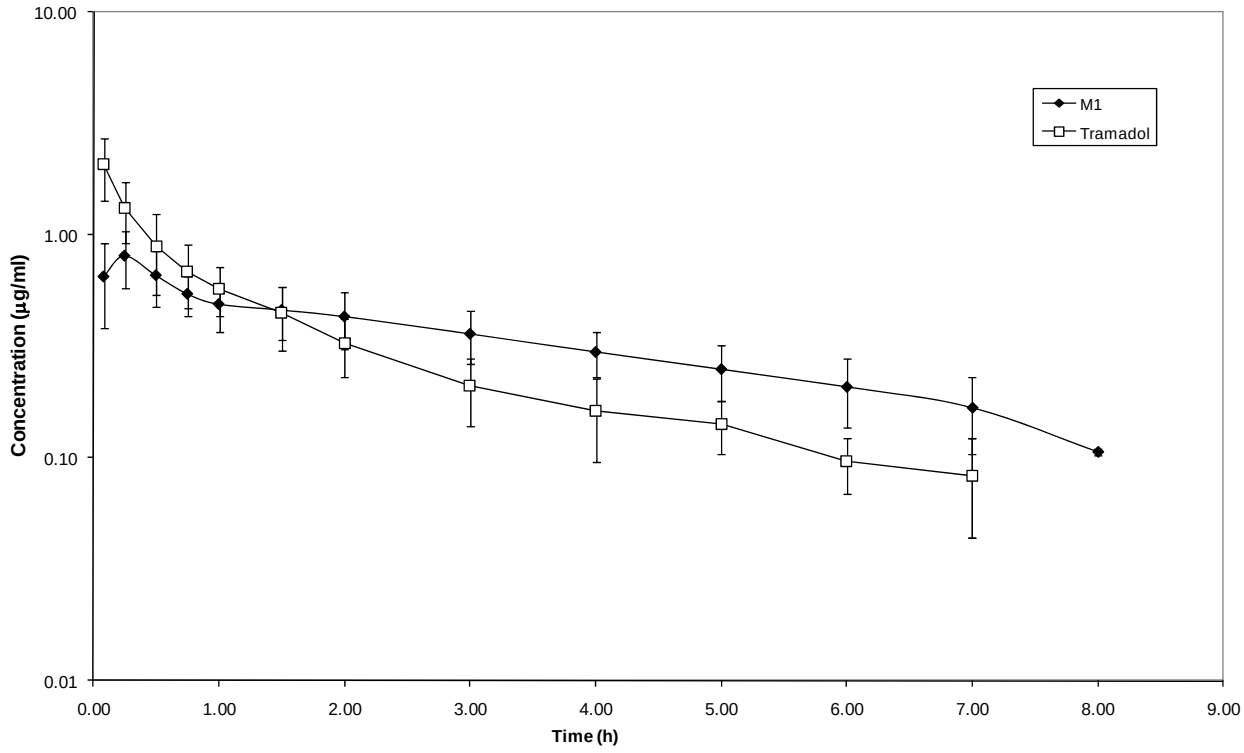


In no case did pain score exceed the level required for the rescue analgesia administration and differences between males and females were never statistically significant. Nevertheless, female cats had a higher pain score at first observation than males (7.5 in females versus 5.7 in males) which decreased rapidly to 2 by 0.5 h. Pain score decreased less rapidly in males, with mean scores of 4.8 at 0.5 h and 3.5 at 1 h. For the remaining postoperative period the two pain score curves were very similar, with scores very close to 2. No side-effects were observed during the observation period.

Serum concentrations

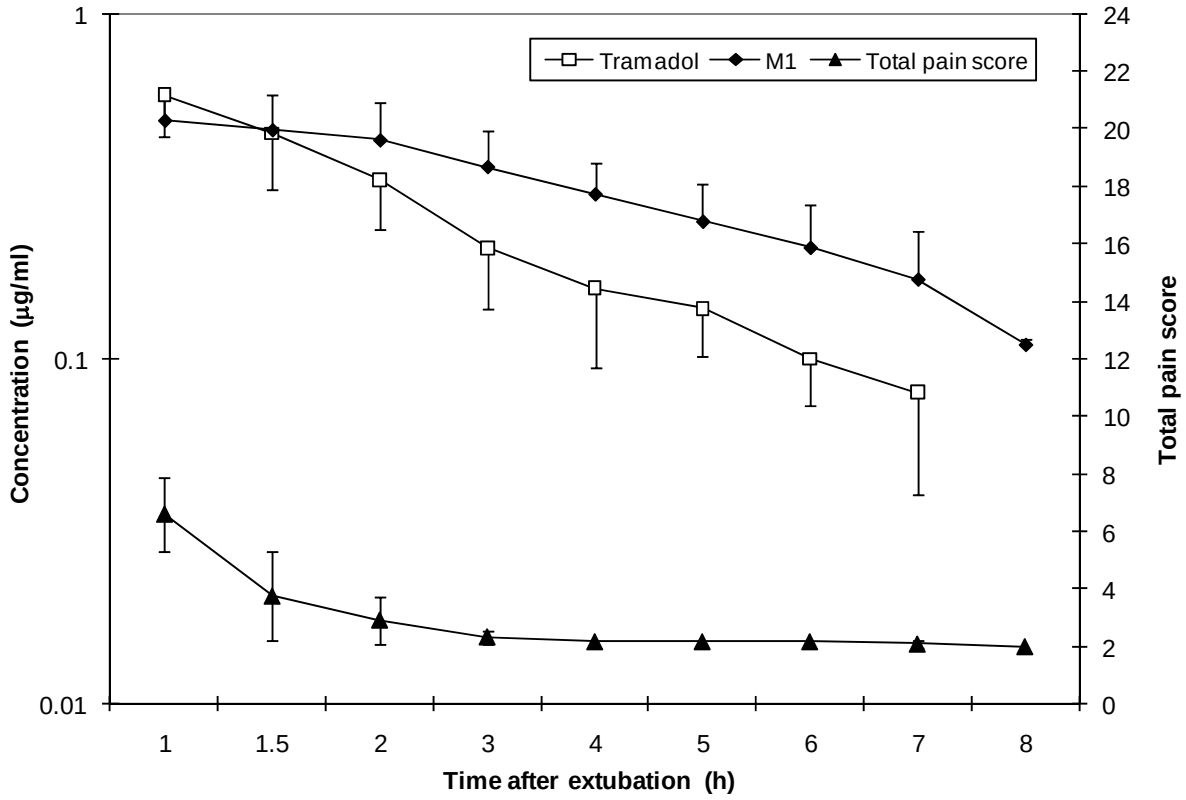
Mean serum concentrations of tramadol and M1 after IV administration are shown in Fig. 2.

Figure 2. Mean (±SD) serum concentrations (μg/ml) of tramadol and M1 in cats (n =8) after IV administration of tramadol at 2 mg/kg



Mean tramadol concentration in serum was 2.08±0.64 μg/ml at first sampling (0.08 h), decreased to 0.58±0.14 μg/ml at 1 h post-treatment, and subsequently declined more slowly to 0.07±0.05 μg/ml at 7 h. The mean peak concentration of M1 (0.81±0.23 μg/ml) occurred at 0.25 h. M1 decline was slower than for tramadol and at last sampling (8 h) was 0.11±0.01 μg/ml, while tramadol was below the limit of quantification (0.05 μg/ml). The mean percentages of protein binding were 15% for tramadol and 17% for M1. In Fig. 3 pain scores and serum concentrations of tramadol and M1 are plotted versus time.

Figure 3. Mean (±SD) serum concentrations (µg/ml) of tramadol and M1 in cats after IV administration of tramadol at 2 mg/kg with mean (±SD) pain score (males + females) plotted versus time after extubation.



Pharmacokinetics

The time courses of tramadol after IV administration was described by a two-compartment open model and a non-compartmental model was applied to M1 serum concentrations. Table 2 shows pharmacokinetic variables for the two sexes separately.

Table 2: Pharmacokinetic parameters (mean±SD) of tramadol and *O*-desmethyl tramadol (M1) after IV administration in 8 cats at the dose of 2 mg/kg

Parameter (units)	**Tramadol** mean ± SD			**M1** mean ± SD		
	Males (n. 4)	**Females (n. 4)**	**Males + Females**	**Males (n. 4)**	**Females (n. 4)**	**Males + Females**
$t_{1/2\square z}$ (h)	n.d.*	n.d.*	n.d.*	3.32±1.18	3.85±1.1	3.54±1.17[a]
AUMC (h.h.μg/ml)	4.46±2.71	8.72±5.05	6.59±4.39	21.88±16.8	23.81±21.02	22.85 ±17.35
$MRT_{(0-\infty)}$ (h)	1.86±0.37	2.94±0.92	2.40±0.87	5.38±2.83	6.07±2.55	5.73±2.82
$AUC_{(0-\infty)}$ (h.μg/ml)	2.25±1.02	2.82±0.71	2.53±0.87	3.72±1.32	3.47±1.44	3.61±1.28
$t_{1/2\lambda1}$ (h)	0.12±0.09	0.21±0.16	0.15±0.12[a]	n.d.	n.d.	n.d.
$t_{1/2\lambda2}$ (h)	1.54±0.4	2.35±0.9	1.86±0.66[a]	n.d.	n.d.	n.d.
C_0 (μg/ml)	2.87±0.74	2.23±0.46	2.60±0.64	n.d.	n.d.	n.d.
V_{dss} (ml/kg)	1831.78±515.26	2075.13±322.54	1953.65±418.68	n.d.	n.d.	n.d.
V_c (ml/kg)	736.59±206.2	844.60±157.38	810.60±187.34	n.d.	n.d.	n.d.
Cl_B (ml/h.kg)	1052.08±473.94	738.53±153.21	895+30±366.62	n.d.	n.d.	n.d.
C_{max} (μg/ml)	n.d.*	n.d.*	n.d.*	0.89±0.31	0.72±0.09	0.81±0.23
T_{max} (h)	n.d.*	n.d.*	n.d.*	0.25±0.0	0.25±0.0	0.25±0.0

$t_{½\lambda z}$ = elimination half-time; AUMC = area under moment curve; $MRT_{(0-\infty)}$ = mean residence time; $AUC_{(0-\infty)}$ = area under serum concentration-time curve; $t_{½\lambda1}$ = distribution half-time; $t_{½\lambda2}$ = elimination half-time; C_0 = serum concentration at time 0; V_{dss} = volume of distribution at steady state; V_c = volume of distribution in central compartment; Cl_B = body clearance; C_{max} = maximum concentration; T_{max} = observed time for C_{max}; [a] harmonic mean ± pseudo SD; n.d. = not done for non-compartmental model; n.d.* = not done for bi-compartmental model

No sex-related differences for these variables were found and therefore, the means for males plus females are reported and discussed. Mean AUC values for tramadol and M1 were 2.53±0.87 h.μg/ml and 3.61±1.28 h.μg/ml, respectively, and mean MRT values 2.40±0.87 h and 5.73±2.82 h for tramadol and M1 respectively. Distribution and elimination half-lives for tramadol were 0.15±0.12 h and 1.86±0.66 h, while the terminal half-life for M1 was 3.54±1.17 h. Tramadol C_0 was 2.60±0.64 μg/ml and M1 C_{max} and T_{max} were 0.81±0.23 μg/ml and 0.25±0, respectively.

Discussion

The purpose of this study was to provide pharmacokinetic data about tramadol and its M1 metabolite in cats administered before surgery at the dose of 2 mg/kg (IV) and to evaluate the clinical efficacy of tramadol as postoperative analgesic.

Tramadol, as used in this study, did not cause respiratory depression but can reduce the isoflurane requirement and produce postoperative analgesia in cat undergoing gonadectomy. No difference in the pharmacokinetic behaviour were detected between sexes.

The dose of tramadol administered in this study (2 mg/kg IV) was chosen on the basis of previous studies on cats (Brondani et al., 2006; Pypendop and Ilkiw, 2008; Brondani et al., 2009a) and in consideration that the veterinary product used (Altadol, Formevet, Italy) is authorized in Italy for use in dogs by IV, IM or oral administration at the same dosage.

Because spontaneous ventilation, normocapnia and high oxyhaemoglobin saturation were maintained throughout the procedure in all animals (Table 1, individual data not shown) we conclude that preoperative IV administration of a single dose of tramadol to cats undergoing elective gonadectomy did not cause clinically significant hypoventilation. Tramadol reduces total ventilatory CO_2 sensitivity by acting on μ opioid receptors in the human brainstem, but does not depress the hypoxic ventilatory response (Grond and Sablotzki, 2004). Postoperative respiratory depression leads to hypercapnia and if severe may also lead to hypoxaemia (Grond and Sablotzki, 2004). Clinical studies in humans indicate absence of significant respiratory depression at analgesic doses of tramadol compared to traditional opioid drugs (Houmes et al., 1992; Vickers et al., 1992; Tarkkila et al., 1997; Tarkkila et al., 1998). This important difference is exploited in pediatric medicine and adults with compromised cardiopulmonary function or contra-indicated for non-opioid analgesics (Grond and Sablotzki, 2004).

The ability of tramadol to reduce the minimum alveolar concentration (MAC) of inhalational anaesthetics has been previously reported in rats (De Wolff et al., 1999) and cats (Ko et al., 2008). In our study, mean end tidal isoflurane was 1.36% (±0.37) with no significant difference between males (1.31% ±0.32) and females (1.47% ±0.43). These concentrations are lower than those suggested as necessary to maintain "surgical" anaesthesia in cats when no analgesics are administered (Steffey and Mama, 2007). However, acepromazine might have contributed to the sparing effect of isoflurane and might have produced an antinociceptive effect as described by Steagall et al. (2008).

Pain assessment in cats is challenging because of the limited information available on the severity of postoperative pain, behavioural indicators of pain and effects of surgery on well-being (Ilkiw, 2003; Robertson, 2005). Further, unlike dogs (Buback et al., 1996; Conzemius et al., 1997; Holton et al., 1998; Firth and Haldane, 1999; Reid et al., 2007), no scales have been validated for

pain assessment in the feline species. However, according to Lascelles and Waterman (1997), observation of behaviour is the best means of assessing the pain experienced by cats. A composite rating scale was adopted by Brondani et al. (2006 and 2009a) where the subcutaneous administration of tramadol (2 mg/kg) in cats provided significantly superior analgesia and decreased the requirement of rescue analgesia compared to placebo. For these reasons we also employed a composite rating scale (Smith et al., 2004) in which comfort, movement, appearance, unprovoked and interactive behaviour and vocalisation were assessed by a single observer every 30 min up to 6 h after extubation. For all animals, scale scores were always below the threshold chosen for the administration of rescue analgesia (Fig. 1) suggesting that tramadol was able to produce sufficient postoperative analgesia in cats undergoing gonadectomy.

Initial tramadol concentrations varied considerably between animals (range 1.2-3.2 μg/ml) and thus high inter-individual variability was found for both tramadol and consequently M1 (C_{max} 0.6-1.3 μg/ml at 0.25 h). Nevertheless M1 concentrations peaked at just under 1 μg/ml at 0.25 h after tramadol administration and remained high (>0.01 μg/ml) for the entire observation period (Fig. 2). Since 0.01 μg/ml is the lowest concentration associated with therapeutic efficacy in humans (Lehmann et al., 1990) this could suggest that M1 has contributed to tramadol analgesia in cats. However, the lowest concentration associated to an analgesic effect in the feline species is unknown and from the results here obtained it is not possible to identify an efficacious serum concentration of M1 in cat (Fig. 3).

At extubation (corresponding to 1 h after tramadol administration) maximum pain was recorded (6.59±1.29) but tramadol and M1 concentrations were already decreased to 0.58±0.14 and 0.49±0.12 μg/ml, respectively. During the rest of the recovery the pain scores were decreased to 2, while tramadol and M1 concentration gradually and constantly decreased to 0.08±0.04 and 0.17±0.06 μg/ml, respectively. The negative correlation between plasma concentration and pain could simply represent an indirect relationship – hysteresis - which cannot be identified with parenteral administration. All the animals in our study were comfortable and they did not require rescue analgesia at any time. The higher pain score soon after extubation might be associated with increased loco-motor activity due to post-anaesthetic excitation or possible euphoria/dysphoria. According to Steagall et al (2008) subcutaneous administration of tramadol (1 mg/kg) produced dysphoric effect in two over eight pain free cats. Further, one of those cats did not become tranquillized even when tramadol was combined with acepromazine. In our study all the animals

underwent surgery; however, it can be speculated that orchiectomy is less invasive and then less painful than ovariectomy. Therefore, same serum concentration of tramadol and M1 might have produced excitatory behavioural effect in male cats but not in females because of different levels of pain. However, no statistically significant differences were detected in the postoperative period between the two gender and none of the cats appeared to be in distress and required sedation or more analgesia. The lack of sensitivity of the scoring system used and the restricted number of animals studied should also be taken into consideration. However, as mentioned above, no pain scoring system has been validated in the feline species yet. Clinical observations and a lack of a need for further analgesic intervention suggest that perioperative tramadol provided analgesia in the postoperative period.

The pharmacokinetic parameters we derived for tramadol differ somewhat to those recently published by Pypendop and Ilkiw (2008) in female cats. Our results showed higher values for tramadol AUC (2.53±0.87 h.μg/ml versus 1.79±0.25 h.μg/ml) and C_0 (2.60±0.64 μg/ml versus 1.3±0.09 μg/ml), and consequently lower values for V_{dss} (1953.65±418.68 ml/kg versus 3000±100 ml/kg) and Cl (895.30±366.62 ml/h.kg versus 1248±192 ml/h.kg). However, pharmacokinetic data for M1 were consistent between the two studies, particularly in terms of M1 concentration that at about 2 h after tramadol administration was higher than the parent compound. The lower clearance of tramadol in our study is likely due to the fact that our cats were anaesthetized and undergoing neutering, while those of Pypendop and Ilkiw (2008) were experimental cats under laboratory conditions; in addition, our animals were less homogenous in terms of weight and age. The fact that Pypendop and Ilkiw (2008) studied only female cats is unlikely to be significant, since our data indicate no sex-related differences in tramadol pharmacokinetics.

Like Pypendop and Ilkiw (2008), we found that the M1:tramadol AUC ratio was >1 in cats, whereas in dogs this ratio is about 0.3 (Kukanich and Papich, 2004; Vettorato et al., 2006, Vettorato et al., 2010), indicating considerably greater M1 production in cats than in dogs, even though the higher AUC of M1 in cats may have been influenced by its slower elimination. In human, the analgesic efficacy of tramadol administration is correlated to the probably synergistic effect of tramadol itself and M1 and the contribution of M1 in cats may deserve an accurate revision. In a recent paper, Pypendop et al. (2009) reported that M1 plays a minor role in thermal analgesia of cats. Further, when administered orally, tramadol doses ≥ 2 mg/kg were required to induce a significant and sustained effect. According to the same authors, a dose of 4 mg/kg administered every 6 h may maintain analgesia close to the tramadol

maximum effect. In our trial, when 2 mg/kg were administered IV, serum tramadol and M1 concentrations were comparable to those obtained by Pypendop et al. (2009) with the oral dose of 4 mg/kg and no respiratory depression was observed. However, a repeated administration study would be advocated to better define an appropriate and long term dosage scheme for tramadol in the feline species.

In humans, M1 seems mainly produced by the 2D6 isoenzyme of cytochrome P-450 (CYP2D6) and its production varies in relation to the presence of different polymorphisms of the isoenzyme (Poulsen et al., 1996). A recent study (Shah et al., 2007) suggested that cytochrome P450 2D activities were similar in dogs and female cats, but lower in male cats, and higher in male and female cats than humans, while Chauret et al. (1997) reported that there were no marked sex-related differences in the metabolism of the different catalytic activity markers tested in human, dog, horse, and cat. Moreover, in our study, although carried out on only four animals of each sex, there were no pharmacokinetic differences between the sexes. The persistent M1 we found in cats could be due to slow glucuronidation and consequently slow M1 elimination, since M1 elimination is reported to require glucuronidation in humans (Overbeck and Blaschke, 1999; Allegaert et al., 2006).

Comparison of our present results with those obtained in dogs using the same tramadol dose (2 mg/kg) under similar conditions (Vettorato et al., 2010) shows that M1 levels were maintained for a longer period in cats than dogs (C_{max} 0.81±0.23 μg/ml versus 0.31±0.17 μg/ml), with mean concentrations of 0.1 μg/ml in cats and 0.05 μg/ml in dogs 8 h after administration, hence greater AUC in cats than dogs (3.61±1.28 h.μg/ml versus 1.24±0.16 h.μg/ml) and a consequent higher M1:tramadol AUC ratio in cats. It is not possible to compare the efficacy evaluations between the two studies due to the differences in surgical procedures (gonadectomy versus tibial plateau levelling osteotomy). Since tramadol administration leads to M1 formation also in the feline species, the potential analgesic effect of M1 in this species needs to be better elucidated.

Conclusions

Preoperative administration of tramadol (2 mg/kg IV) to 12 cats undergoing gonadectomy did not cause clinically significant hypoventilation, decreased the isoflurane requirement and, according to the pain scoring system used, produced sufficient postoperative analgesia. These findings, together with the positive kinetic behaviour, suggest that 2 mg/kg of tramadol IV might be useful as intra and postoperative analgesic in cat sedated with acepromazine and

undergoing gonadectomy. However, further studies are advocated to better understand the analgesic property and the appropriate dosage of tramadol in the feline species.

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CHAPTER 4

Pharmacokinetics, intraoperative effect and postoperative analgesia of tramadol in horses undergoing orchiectomy

Very few drugs are available for analgesia in horses. Alpha-2 adrenergic agonists and nonsteroidal anti-inflammatory drugs are used mostly for acute and visceral pain but the former can cause considerable sedation at doses used for analgesia (Pippi and Lumb, 1979; Muir and Robertson, 1985). Moreover non steroidal anti-inflammatory drugs have some potential adverse effects and opioid analgesics, except butorphanol, are not commonly used because substantial sympathetic stimulation and excitation of the central nervous system (CNS) are observed when used IV (Natalini and Robinson, 2000). Although tramadol is authorized for use in dogs in Italy, it has not been approved for use in horse. The pharmacokinetic studies recently carried out by some authors are quite conflicting (Zonca et al., 2006; Giorgi et al., 2007; Shilo et al., 2008, Dhanjal et al., 2009; Cox et al., 2010), while only one study about clinical efficacy is available (Fonda, 2002), although these could represent a valid tool to clarify the role of tramadol in horse.

The present study reports (a) the serum pharmacokinetic profile and urinary excretion of tramadol and its M1, M2 and M5 metabolites in horse after IV administration at 4 mg/kg prior to orchiectomy, and (b) a clinical evaluation of the efficacy of tramadol as postoperative analgesic. Aim of this study was to generate data for the rational dosing of this substance in the horse.

Materials and methods

Animals

The study was performed on 8 healthy colts (6 Arabian horses, 1 Thoroughbred and 1 Quarter Horse cross breed), 1-2 years old, weighing between 292 kg and 490 kg, undergoing orchiectomy. All animals came from the same farm, were bred at pasture and not backed, thus were kept for 4 days in barn of the hospital before surgery. All animals were judged healthy (ASA status I) on the basis of physical examination and results of routine blood tests, and were enrolled in the study after written consent from the owner, as required by Italian law (D.L. 116/1992). The study protocol was approved by the Ethical Committee of the University of Milan.

Anaesthetic and surgical procedures

All animals received acepromazine maleate (0.05 mg/kg, Prequillan, Fatro) and detomidine (0.015 ± 0.005 mg/kg, Domosedan, Pfizer) intramuscularly (IM), as pre-anaesthetic medications. Anaesthesia was induced by IV ketamine (2.2 mg/kg, Ketavet, Intervet) and diazepam (0.05 mg/kg, Diazepam, Intervet) mixed in the same syringe. After intubation anaesthesia was maintained with isoflurane in oxygen (100%) in intermittent positive-pressure ventilation (IPPV)

to maintain end-tidal carbon dioxide values between 39 and 42 mmHg. Tramadol (4 mg/kg, Altadol, Formevet) was administered IV as a bolus over 60 seconds through a jugular catheter (14 gauge), 5 min after intubation and 15 min prior to beginning surgery. During surgery lactated Ringer's solution was administered at 3 mL/kg/h through the same catheter. During anaesthesia, variations of isoflurane concentration were performed to maintain an appropriate depth of anaesthesia based on clinical assessment; signs monitored included degree of nystagmus, movement, muscle relaxation, response to surgery, invasive blood pressure (IBP), heart rate (HR). All horses underwent orchiectomy according to standard surgical procedures. During surgery, IBP, HR electrocardiogram (lead II), oxyhaemoglobin saturation (SpO_2), end tidal carbon dioxide ($EtCO_2$), invasive systolic arterial pressure (SAP), invasive mean arterial pressure (MAP) and invasive diastolic arterial pressure (DAP) were recorded every 5 min using a UT4000F Pro monitor (Goldway Inc.).

After extubation postoperative pain assessment was performed by observations of pain responses (yes vs no: signs of pain present vs absent) together with assessment of the severity of pain. The severity of pain was evaluated by a visual analogue scale (VAS, Hubbel, 1999) that provides a semiobjective scoring method for evaluating pain in horses. Pain was assessed at extubation (0) and at 0.5, 1, 2, 3, 4, 6, 8, and 12 h. The trained evaluator places a time-dated mark on a 10 cm line, where 0 cm refers to absence of pain and 10 cm worst possible pain. For this study "No pain" was considered in the VAS from 0 to 3 cm, "Moderate pain" was considered from 3 to 6 cm and "Worst pain" was considered from 6 to 10 cm.

Pain was judged to be unacceptable if a score ≥ 5 cm was awarded using VAS. The "rescue analgesia" protocol was 0.1 mg/kg of butorphanol (Dolorex, Intervet) IV. In case of a second evaluation of a pain score ≥ 5 cm, 1 mg/kg of flunixin meglumine (Alivios, Fatro) was administered IV.

At 12 h after extubation, 1 mg/kg of flunixin meglumine was administered IV in all horses to control signs of inflammation.

Collection, purification and analysis of serum and urine samples

Due to the dangerous recalcitrance of the horse n. 4, it was not possible to obtain blood sample after recovery and thus this subject was excluded from kinetic analyses.

For all the other 7 animals, venous blood samples (10 mL) were collected from a jugular vein catheter into non-heparinized tubes before tramadol administration (time 0) and at 5, 10, 20, 30, 45 min and 1, 1.5, 2, 4, 8, and 10 h

after tramadol administration. Urine samples were collected after spontaneous urination using specific collection bags, each 12 hours or when full, for a maximum of 3.5 days after treatment. All samples were centrifuged (1500 g, 10 min at room temperature for serum and 3500 g for 5 min for urine) soon after collection and stored at –80°C pending assay.

Serum and urine samples were purified by solid phase extraction and residues of tramadol, M1, M2 and M5 were analysed by HPLC with the method reported in the paper by Cagnardi et al. (2010).

Solutions for the calibration curve were prepared diluting stock solutions of tramadol, M1, M2 and M5 (1 mg/mL) to obtain concentrations in the range 0.02 to 5 μg/mL and 0.02 to 10 μg/mL in blank horse serum and urine, respectively.

Serum binding of tramadol, M1, M2 and M5 in the range 0.1-20 μg/mL was determined in vitro. The serum-bound molecules were removed by ultrafiltration (Villa et al., 1997) using a disposable device (Amicon, Millipore) and free substances in the filtrate were analyzed by HPLC as described above.

Tramadol hydrochloride, M1, M2 and M5 were purchased from LGC Standards. Other reagents and solvents were purchased from J.T. Baker.

Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 5.2.1 software (Pharsight Corporation, USA) which allows compartmental and non compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model that best fitted the data. All data points were weighted by the inverse square of the fitted value. Serum concentrations after IV tramadol administration were fitted to a standard bi-exponential curve (Gibaldi and Perrier, 1982) describing a two-compartment model with elimination from the central compartment.

Parameters estimated from the model were used to calculate pharmacokinetic variables for each animal. The volume of distribution in the central compartment (V_c) was calculated as:

$$V_c = Dose/C_0$$

where *Dose* is dose of tramadol and C_0 is the extrapolated serum concentration of tramadol at time 0. The kinetics of M1, M2 and M5 were determined by non-

compartmental analysis. Mean residence time (MRT), body clearance (Cl_B) and volume of distribution at steady state (V_{dss}) were determined from the following equations (Gibaldi and Perrier, 1982):

$$MRT = AUMC/AUC$$
$$Cl_B = Dose/AUC$$
$$V_{dss} = Cl_B * MRT$$

where *AUMC* is area under the moment curve and *AUC* is area under serum concentration-time curve.

Statistical methods

Means and standard deviations (SD) of intraoperative variables and pain scores were calculated. Pharmacokinetic parameters were reported as means (SD), harmonic means with pseudo-standard deviations were calculated for half lives using a jack-knife technique (Lam et al., 1985).

Results

Intra-anaesthetic evaluation and postoperative analgesia

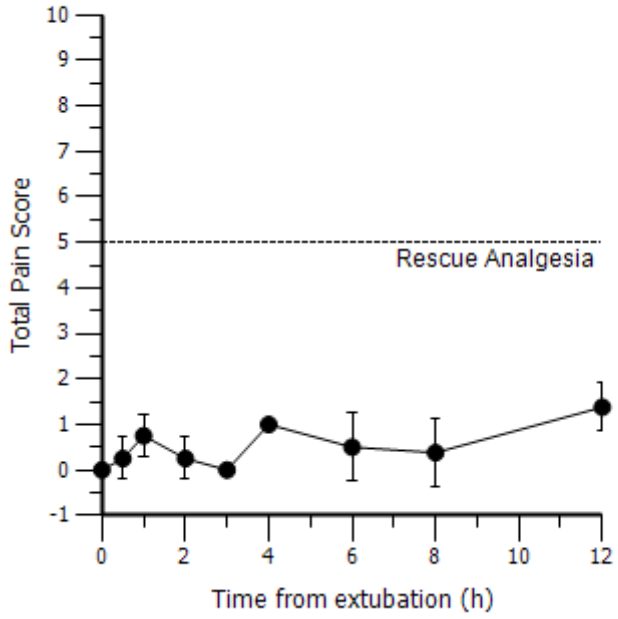
No adverse effects were observed after IV tramadol administration during the whole observation period. Mean age, body weight, duration of surgery, duration of anaesthesia, HR, invasive systolic arterial pressure (SAP), invasive mean arterial pressure (MAP), invasive diastolic arterial pressure (DAP), SpO_2 and $EtCO_2$ are shown in Table 1.

Table 1. Mean (±SD) values of general characteristics, selected surgical variables, and subjective pain score in 8 horses undergoing surgical gonadectomy

	Mean ± S.D. (n. 8)
Age (months)	29 ± 8.7
Body weight (kg)	351.7 ± 70.2
Anaesthesia Time (min)	47.1 ± 2.17
Surgery time (min)	19.4 ± 1.19
Time from tramadol injection to start of surgery (min)	15
Heart rate (per minute)	36.8 ± 4.23
Invasive systolic arterial pressure (SAP)	111.9 ±9.8
Invasive mean arterial pressure (MAP)	89.7 ±12.5
Invasive diastolic arterial pressure (DAP),	79.4 ± 12.5
End tidal CO_2 (mmHg)	40.45 ± 1.2
Oxyhaemoglobin saturation (%)	98.6 ±0.6
Subjective pain score	0.5 ± 0.6

The results of the subjective pain evaluation over the 12 h after extubation are shown in Fig 1. Pain score was max 2 cm in any observation time and no animal required rescue drugs.

Figure 1. Total score of postoperative pain evaluations in the horses (n = 8) assessed at various times after extubation using the visual analogue scale.

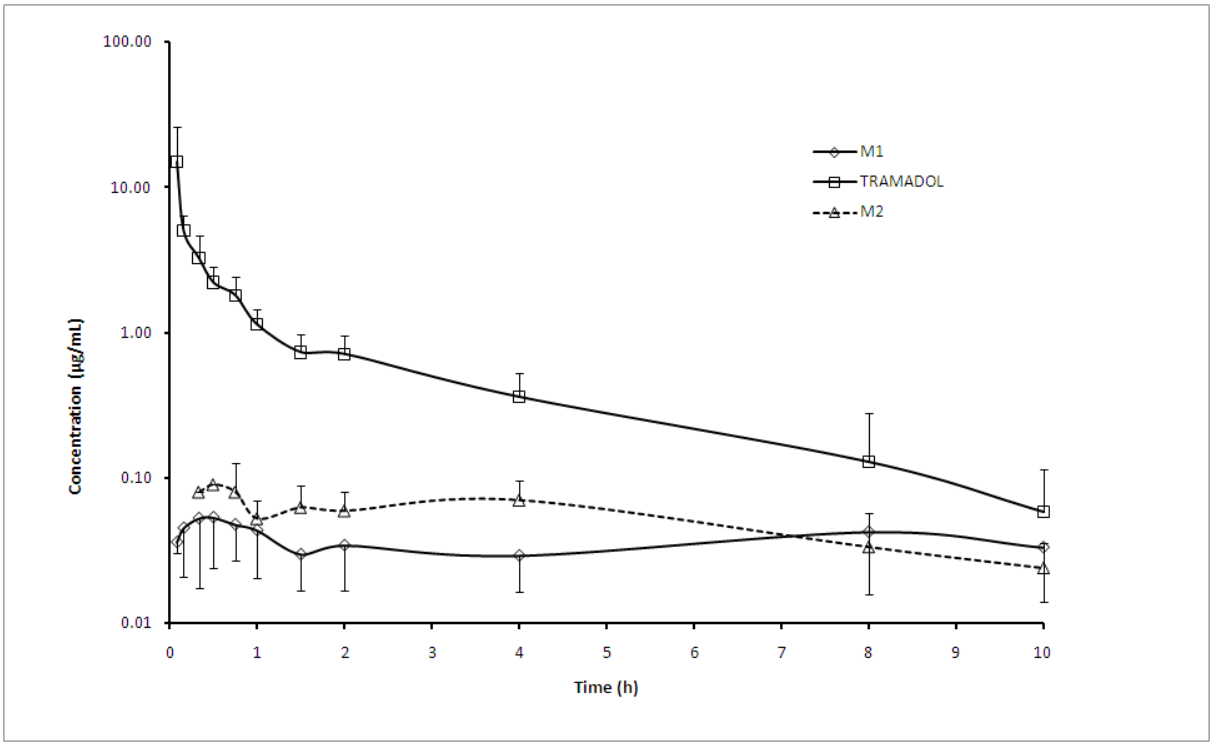


Serum and urine concentrations

The HPLC methods in serum and urine were found to be linear in the range 0.02-5 μg/mL and 0.02-10 μg/mL, respectively. Intra-laboratory investigation indicated limits of quantification (LOQ) of 0.02 μg/mL for all compounds in both matrices, while in serum the limits of detection (LOD) were 0.0009 μg/mL for tramadol, 0.002 μg/mL for M1, 0.0008 μg/mL for M2 and 0.001 μg/mL for M5 and in urine were 0.002 μg/mL for tramadol, 0.003 μg/mL for M1, 0.001 μg/mL for M2 and 0.002 μg/mL for M5.

Mean serum concentrations of tramadol, M1 and M2 after IV administration are shown in Fig. 2. In all the serum samples M5 was never detected (<LOD).

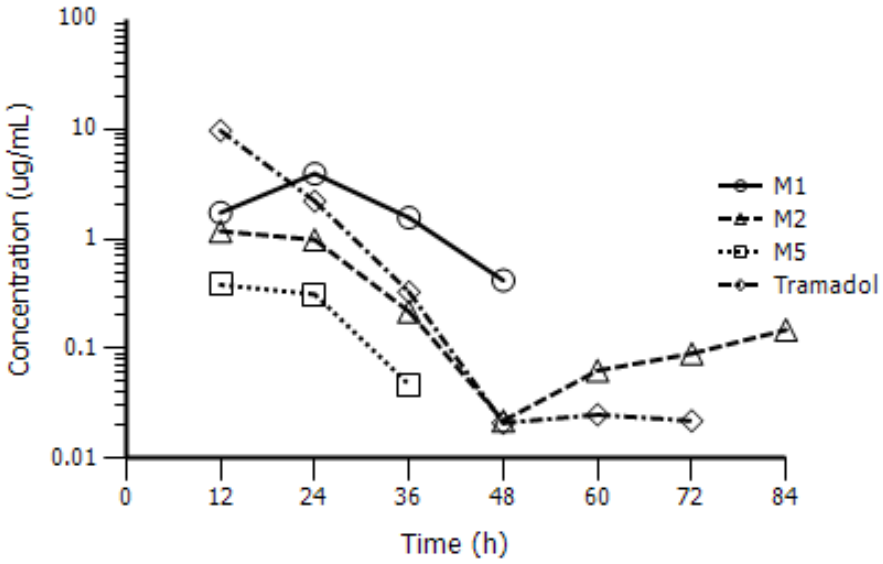
Figure 2. Mean (SD) serum concentrations (μg/mL) of tramadol, M1 and M2 in horses (n =7) after IV administration of tramadol at 4 mg/kg.



Mean tramadol concentration in serum was 14.87±11.14 μg/mL at first sampling (0.08 h), decreased to 1.13±0.31 μg/mL at 1 h post-treatment, and subsequently declined more slowly to 0.05±0.06 μg/mL at 10 h. M1 was detected at the first sampling point (0.08 h) only in two horses with a mean value of 0.036±0.01 μg/mL, at 0.16 h 5 horses presented quantifiable concentrations with a mean value of 0.045±0.02 μg/mL, from 0.33 h M1 was always detected in all horses. From this time point (0.33 h) to 0.75 h M1 concentrations reached a peak plateau of about 0.05 μg/mL that declined little to 0.033±0.02 μg/mL at 10 h. M2 concentrations were below the LOQ at 0.08 and 0.16 h, these were detected only in 1 horse at 0.33 h and 0.5 h (0.08 and 0.09 μg/mL, respectively), at 0.75 h M2 was detected in 3 horses out of 7 with mean concentrations of 0.08±0.05 μg/mL, at 1 h 4 horses had quantifiable mean concentrations of 0.052±0.02 μg/mL and at 2 h all horses showed detectable concentrations with a mean value of 0.059±0.02 μg/mL. At 10 h M2 levels were quantified in 4 horses with a mean value of 0.025±0.01 μg/mL. The mean percentages of protein binding were 19.5% for tramadol, 14.7% for M1, 26.1% for M2 and 18.7% for M5.

Urine samples were collected after spontaneous urination in collection bags that were emptied each 12 hours or when full. The complete urine collection of 3.5 days was obtained only from 5 horses. The samples were analysed to detect the excretive profile of tramadol and its metabolites. The profile observed was rather variable and thus it was not possible to carry out a kinetic analysis. Tramadol concentrations were above LOQ until 36 h after administration in all horses and only in 1 horse (n.7) was quantified until 72 h. M1 was eliminated until 24 h in 1 horse (n.3), until 36 h in other 2 horses (n.5 and 8) and until 48 h in the remaining 2 (n.1 and 7). M2 was present until 24 h in 3 horses (n. 1, 5 and 8) and in the other 2 horses until 36 h (n.7) or 86 h (n.3). M5 was recovered until 12 h in 1 horse (n.1), until 24 h in another horse (n.3) and until 36 h in the rest 3 horses (n.5, 7 and 8). The urinary concentration time profile of tramadol and its metabolites is shown in Fig. 3. The most recovered compound was tramadol, followed by M1, M2 and M5.

Figure 3. Mean urinary concentrations of tramadol, M1, M2 and M4 in 5 horses after tramadol IV administration at 4 mg/kg



Pharmacokinetics

The time courses of tramadol and its metabolites (M1 and M2) serum concentrations after IV administration were best described by a two-compartment open model and a non-compartmental model, respectively. The results are summarized together in Table 2.

Table 2. Pharmacokinetic parameters [mean±SD (range)] of tramadol, *O*-desmethyl tramadol (M1) and *N*-desmethyltramadol (M2) after IV administration in 7 horses at the dose of 4 mg/kg

Parameter (units)	Tramadol mean ± SD (range)	M1 mean ± SD (range)	M2 mean ±SD (range)
$t_{1/2\lambda z}$ (h)		5.59±19.6[a, b](1.04-74.34)	3.87±2.48 [a, c] (1.97-6.36)
$AUMC_{(0\text{-last})}$ (h.h.μg/mL)	12.11±6.72 (6.79-13.61)	1.50 ±1.13 (0.08-3.39)	1.78±1.28 (0.36-3.08)
$MRT_{(0\text{-last})}$ (h)	1.48±0.62 (0.63-2.10)	4.15±1.54 (1.05-5.60)	3.75±1.01 (2.07-4.89)
$AUC_{(0\text{-last})}$ (h.μg/mL)	9.03±4.76 (17.69-4.19)	0.31±0.19 (0.08-0.64)	0.43±0.23 (0.12-0.76)
$t_{1/2\lambda 1}$ (h)	0.07±0.11[a] (0.02-0.36)		
$t_{1/2\lambda 2}$ (h)	2.1±0.55[a] (1.73-3.88)		
C_0 (μg/mL)	78.63±148.72 (5.49-409.31)		
V_{dss} (mL/kg)	1141.91±578.43 (166.52-1632.2)		
V_c (mL/kg)	332.43±260.52 (9.77-728.32)		
Cl_B (mL/h.kg)	562.23±270.96 (209.53-695.48)		
C_{max} (μg/mL)	14.87±11.14 (4.88-33.29)	0.06±0.03 (0.04-0.13)	0.09±0.03 (0.05-0.13)
T_{max} (h)		1.69±2.79 (0.33-8)	2.46±1.49 (0.75-4)

$t_{½\lambda z}$ = elimination half-time; AUMC = area under moment curve; $MRT_{(0\text{-last})}$ = mean residence time; $AUC_{(0\text{-last})}$ = area under serum concentration-time curve; $t_{½\lambda 1}$ = distribution half-time; $t_{½\lambda 2}$ = elimination half-time; C_0 = serum concentration at time 0; V_{dss} = volume of distribution at steady state; V_c = volume of distribution in central compartment; Cl_B = serum clearance; C_{max} = maximum concentration; T_{max} = observed time for C_{max}; [a] harmonic mean ± pseudo SD; [b] calculated on 6 horses; [c] calculated on 5 horses

Mean AUC values for tramadol, M1 and M2 were 9.03±4.76 h.μg/mL, 0.31±0.19 h.μg/mL and 0.43±0.23 h.μg/mL, respectively, and mean MRT values were 1.48±0.62 h, 4.15±1.54 h and 3.75±1.01 h for tramadol, M1 and M2 respectively. Distribution and elimination half-life for tramadol were 0.07±0.11 h and 2.1±0.55 h respectively, while the terminal half-life for M1 was 5.59±19.6 h calculated on 6 horses and for M2 was 3.87±2.48 h calculated on 5 horses. M1 and M2 C_{max} and T_{max} were 0.06±0.03 μg/mL and 1.69±2.79 h and 0.09±0.03 μg/mL and 2.46±1.49 h, respectively.

Discussion

The tramadol dose (4 mg/kg) was adopted on the base of previous studies performed with 2.5 mg/kg IV, where a very small production of M1 in horses compared to other species was reported (Zonca et al., 2006), and with 5 mg/kg IV, where adverse effects as tremors and fasciculation were observed (Cagnardi, unpublished data). No recommended dose is authorised in horse.

All the monitored physiological parameters were stable during surgery and this can be attributed to the adequate analgesic management used. IPPV and normocapnia were maintained throughout the procedure in all animals (Table 1).

In our study, tramadol may have contributed in reducing the isoflurane requirement, but intraoperatively it was not possible to distinguish its analgesic effect from that of detomidine (Mama et al., 2009).

Compared to other species few studies have been carried out to assess objectively and subjectively clinical pain behaviour in horse and no sensitive scoring system has been developed for evaluation of postoperative pain in this species (Price et al., 2003). In our study VAS was used to quantify the severity of postoperative pain in colts, as the signs evaluated during VAS are likely to be indicative of postoperative pain in horse (Price et al., 2003). Moreover VAS are commonly used in human surgery to evaluate postoperative pain associated with various treatment regimens. In addition to its simplicity, the main advantage of VAS is its ability to track trends, if the same evaluator is assessing pain (Mich and Hellyer, 2008). Although this pain assessment scale has been successfully used in human medicine, it relies on a patient's self-report of pain, thus making it a somewhat objective, reliable, and repeatable assessment. In animals, attempts to determine the degree of pain and the ability of the animal to cope with pain are much more difficult. In veterinary medicine, the VAS has also been proven to be sensitive and reproducible (Macdonald et al., 2002; Farstvedt and Hendrickson, 2005), but because it is based on observations rather than direct

verbal or written communication with the patient, it is considered a more subjective measure of pain. In a recent paper by Vettorato et al. (2010b) four recovery quality scoring systems, including VAS, were critically reviewed for reliability in horse and the result was that all four systems studied were similar.

In our study, the overall VAS scores were notably low (≤ 2 cm), i.e. all horses had essentially no signs of pain after the surgical procedure.

Preoperative administration of tramadol to horses undergoing orchiectomy under general anaesthesia showed low postoperative pain score in VAS with no requirement of rescue analgesia up to 12 h. These results suggest that tramadol was able to produce good postoperative analgesia in these horses.

At the first time point (0.08 h) tramadol concentrations varied considerably between animals (range 4.88-33.29 μg/mL), whereas from the second time point more homogenous results were obtained. The high variability observed was also reflected in the metabolites production. Except for M5, that was never detected in serum, M1 and M2 were found variably in all subjects. In general M1 was detected earlier, mainly starting from 0.16 h, while M2 later, starting from 1h. This reflects also on M1 and M2 C_{max} and T_{max}: in fact maximum concentrations of M1 were slightly lower and obtained a little earlier (0.06 ±0.03 μg/mL at 1.69 ± 2.79 h versus 0.09 ± 0.03 μg/mL at 2.46 ± 1.49 h). The large variability observed was quite unexpected considering that the selected animals were homogenous for breed, sex, age and breeding farm. The difference in the metabolite production could be attributed to the immature metabolic pool of each subject due to the young age (1-2 years old) and to inter-individual variability. As reported by Fink-Gremmels (2008), total CYP450 content in young animals is much lower than that of other herbivorous species and moreover CYP3A activity exhibits an age dependent increase of more than 50% when young animals (0–1 year) are compared to adults of more than 12 years. M1 production in humans is mainly due to the CYP2D6 activity and it varies in relation to the presence of different polymorphisms of the isoenzyme, whereas M2 is produced by CYP2B6 and CYP3A (Poulsen et al., 1996). In horse, a study reporting a comparative expression of CYP in the liver, showed that the CYP2D6 amount is low if compared with CYP2B6 and 3A in this animal species (Nebbia et al., 2003).

Tramadol and all metabolites (including M5) were detected in urine, even though significant variability characterised both the amounts and duration of excretion. This variability could be attributed to the immature renal and metabolic functions of our young horses and also to the concomitant administration of other drugs that could interfere with the excretion of tramadol

and its metabolites. This aspect was not further investigated as beyond the aim of this study that was to present only an indicative excretion of these compounds.

The pharmacokinetic parameters we derived for tramadol, M1 and M2 differ somewhat to those published by the other authors at the dose of 2 mg/kg IV (Shilo et al., 2007; Dhanjal et al., 2009) or 5 mg/kg IV (Giorgi et al., 2007). The elimination half-life of tramadol (2.1 ± 0.55 h) was very similar to Dhanjal et al. (2009), but longer than that published previously (0.69 h: Giorgi et al. 2007; 1.4 h: Shilo et al. 2008). This elimination half-life was similar to that reported in dogs (Vettorato et al., 2010a) and cats (Cagnardi et al., 2010), but considerably shorter than that in humans (Murthy et al. 2000). Apparent volume of distribution (1.14±0.58 L/kg) in the present study was smaller to that reported previously by Shilo et al. (2008) (2.17 ± 0.52 L/kg) and by Dhanjal et al. (2009) (2.48±0.74 L/kg). Clearance of tramadol in the present study was 9.37 ± 4.52 mL/min/kg, lower than those reported following a 2 mg/kg dose by Shilo et al. (2008) (26 ± 3 mL/min/kg) and by Dhanjal et al. (2009) (20 ±6 mL/min/kg), but higher in comparison with the clearance reported by Giorgi et al. (2007) (1.16 mL/min/kg) following a dose of 5 mg/kg . The most relevant difference obtained in our study is the metabolites production. As M1 production was high we were able to carry out a kinetic analyses, conversely to other authors after IV administration (Giorgi et al., 2007; Shilo et al., 2008). M2 production was higher than that of M1, but lower to that observed by Giorgi et al. (2007). We found that the M1:tramadol AUC ratio was >0.03 in horses, whereas in dogs this ratio is about 0.3 and >1 in cats (Vettorato et al., 2010a and Cagnardi et al., 2010, respectively), indicating considerably lower M1 production in horse than in cats and dogs, while M2:tramadol AUC ratio was >0.05, indicating a slightly greater M2 production in horses. MRT was longer for M1 and M2 compared to tramadol, and also elimination half-lives for M1 and M2 were longer, although highly variable and not calculated in all horse. To explain the differences we obtained both in tramadol and metabolites kinetics it has also to be stressed that our horses underwent anaesthesia and were administered with other drugs that could interact with the metabolism and elimination of tramadol and its metabolites. Furthermore these differences could be attributed, as previously stated about the excretion of this compounds, to the young age of our horses and a consequent immature renal function and metabolic capacity of the liver.

Pharmacokinetic–pharmacodynamic data for tramadol and M1 associated with a clinical response in horses is not available in the literature yet. In humans, the reported minimally effective analgesic concentrations for tramadol varies considerably from 0.02–0.986, 0.65–2.169 and 0.272–1.9 μg/mL (reported by

various authors and reviewed by Grond and Sablotzki, 2004) and those of M1 from 0.36 to 0.84 μg/mL (Grond and Sablotzki, 2004). The ranges of C_{max} for tramadol (4.88-33.29 μg/mL) and M1 (0.04-0.13 μg/mL) in our horses are higher to the analgesic concentrations of tramadol but not of M1. No study has been reported about M1 contribution to analgesic efficacy of tramadol in horses, but considering the positive results about the clinical efficacy of tramadol in our study, it could be plausible that the contribution of M1 in the analgesic efficacy in the horse is quite negligible. Moreover all the others studies performed in the horse recorded a M1 scarce production (Giorgi et al., 2007; Shilo et al., 2008; Dhanjal et al., 2009; Cox et al., 2010), only Dhanjal et al. (2009) studied the antinociceptive effect of tramadol and at the dose of 2 mg/kg it was quite unsatisfactory, while in our study at the dose of 4 mg/kg the clinical efficacy was positive (VAS score ≤ 2). As also suggested by Cox et al. (2010), doses greater than 2 mg/kg would be necessary to achieve targeted plasma concentrations associated with analgesia in humans .

In our study no adverse effects were observed, while administration of 5 mg/kg IV has been reported to cause tremor, confusion, agitation, and tachycardia (Giorgi et al., 2007, Cagnardi unpublished data). In these horses drug administration was carried out slowly over 60 seconds, before surgery, when the animals were already under anaesthesia, thus the tremors or fasciculation were probably hidden. However, all the physiological parameters monitored during surgery did not change after tramadol administration.

Conclusions

Preoperative administration of tramadol (4 mg/kg IV) to 8 horses undergoing orchiectomy did not cause side effects and, according to the visual analogue scale used, produced sufficient postoperative analgesia for 12h postoperative observation time. These findings, together with the positive kinetic behaviour, suggest that 4 mg/kg of tramadol IV might be useful as intra and postoperative analgesic in horses undergoing orchiectomy.

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CHAPTER 5

Pharmacokinetics and efficacy of ketorolac in dogs undergoing surgery

Due to its favourable and potent anti-inflammatory and analgesic activities, KET could represent a useful tool to control acute pain also in animals, such as in the post-operative period. However, the drug is not currently approved for use in veterinary practice. The aim of this study was to determine the pharmacokinetics of KET after a single dose (0.5 mg/kg, IV) and to evaluate its analgesic efficacy in the treatment of post-surgical pain in dogs.

Materials and methods

Animals

Fifteen dogs (5 males and 10 females) aged 0.4-9 years, weighing between 5 kg and 35 kg, were included in the study. All animals were admitted to the Department of the Clinical Veterinary Sciences, University of Milan, for routine surgery and were judged to be healthy (ASA status I) on the basis of physical examination and results of routine blood tests. The study was approved by the Ethical Committee of University and all animals were enrolled after written consent by the owner.

Pre-surgical, surgical and post-surgical procedures

The same anaesthetic protocol was administered in all dogs: pre-medication by atropine sulphate (0.03 mg/kg, IM, Atropina solfato, ATI) and acepromazine (0.05 mg/kg, IM; Prequillan, Fatro) and induction by IV bolus of propofol (4-8 mg/kg, Rapinovet, Intervet) administered to effect. The anaesthesia was maintained by isoflurane (Isoba, Schering-Plough) in 100% oxygen in spontaneous ventilation.

KET (Toradol, Recordati) was administered as IV bolus at the dose of 0.5 mg/kg after intubation and 20 min prior surgery.

The dogs underwent ovariectomy or orchiectomy according to standard surgical procedures. All the surgeries were performed by the same surgeon. Due to the different kind of invasive surgery, in order to recognize possible differences, all evaluations were recorded according to the sex of animals.

During surgery, heart rate, electrocardiogram (lead II), respiration rate, oxyhaemoglobin saturation, end tidal carbon dioxide (Et-CO_2), end tidal isoflurane (Et-Iso) concentrations and minimal alveolar concentration (MAC%) of Isoflurane were recorded every 5 min with Goldway monitor (UT 4000 Fpro).

To evaluate postoperative pain the subjective scores were assessed by a trained observer using a method modified by Smith *et al.* (2004). This scale

provides the assignment of a score for each parameter considered (comfort, movement, appearance, unprovoked behaviour, interactive behaviour and vocalization), with a total result ranging from 0 to 24. Pain was assessed starting from extubation (t = 0) at 15, 30, 45, 60 min, then each hour up to 12 h and at 24 hours. Buprenorphine (10 μg/kg IM) was administered if the pain score was 9 or above.

Samples collection and KET extraction

In 10 dogs (4 males and 6 females) blood samples were collected at t0 (before KET administration, after induction) 5, 15, 30, 45, 60, 90 min and at 2, 3, 4, 6, 8, 10 and 24 hours after administration of KET. Each sample was centrifuged at 1500 g for 10 min soon after collection and the serum was stored at -20°C until analysis.

Serum samples were purified by solid phase extraction by using cartridges (Waters Sep-Pak C18) previously activated with 5 mL of methanol and 10 mL of HPLC water at pH 2.75 adjusted with ortophosphoric acid (acidic water). One mL of sample was acidified with 100 μL of 0.5 M sodium acetate (adjusted to pH 3.0 with glacial acetic acid), vortexed and then diluted with 2 mL of acidic water. Subsequently samples were percolated onto the cartridges, washed with 2 mL of acidic water and with 5 mL of hexane. The extracted were eluted with 8 mL of diethyl ether and reduced to dry residue by rotor evaporator. The samples were then redissolved in 100 μL of acetonitrile in acidic water (50:50) (Pasloske et al., 1999).

High performance liquid chromatography

The serum quantification of KET was performed by HPLC system that included a binary pump, an autosampler, a Peltier column oven set at 20°C and an UV/Visible detector (Series 200, Perkin Elmer, Italy) set at 313 nm of wavelength.

The drug separation was achieved by Zorbax column SB C18 (150x4.6 3.5 μm, Agilent Technologies, USA) whit adequate pre-column. The mobile phase was acidic water and acetonitrile (66:34, v:v) with a flow rate of 1.4 mL/min.

The analytical method was validated intra-laboratory. The calibration curve was prepared with spiked solutions obtained diluting the original stock solution of KET (1 mg/mL) in dog blank serum to achieve concentrations ranging from 0.01 to 10 μg/mL.

The analytical standard of KET tromethamine (purity grade 99%) was provided by Sigma Aldrich (Italy). All reagents and solvents were purchased from Panreac Sa (Italy).

The percentage of KET serum protein binding was assayed *in vitro* with ultrafiltration unit (Amicon Ultrafree MC, Centrifugal Filter Unit, Millipore, USA), according to Villa et al. (1997). Blood samples spiked with KET concentrations ranging from 10 to 50 μg/mL were centrifuged at 4500 *g* for 30 min with 30000 Nominal Molecular Weight Limit (NMWL) cut-off ultrafiltration units and injected in HPLC system. The binding percentage was calculated by serum (unbinding drug) and methanol (total KET) peak area ratio.

Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 5.2.1 Prof software (Pharsight Corporation, USA) which allows compartmental and non-compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value.

The disposition of ketorolac following IV administration in this study was described by either two compartments (seven dogs) or three compartments model (three dogs) depending on the individual animal. Non compartmental analysis (NCA) was also performed.

In compartmental kinetic KET distribution and elimination were well described by the following equations:

$$C_{(t)} = Y_1 {}^{(-\lambda 1 t)} + Y_2 {}^{(-\lambda 2 t)}$$
$$C_{(t)} = Y_1 {}^{(-\lambda 1 t)} + Y_2 {}^{(-\lambda 2 t)} + Y_3 {}^{(-\lambda 3 t)}$$

where $C_{(t)}$ (μg/mL) is serum drug concentration at time *t*; Y_1, Y_2 and Y_3 are serum concentrations extrapolated to time zero of the drug distribution and elimination phases (in bi-compartmental or tri-compartmental models); λ_1, λ_2 and λ_3 are the slopes of the distribution and elimination phases of the drug, respectively. The distribution half-life ($t_{1/2\lambda 1}$) and terminal half-lives ($t_{1/2\lambda 2}$ and $t_{1/2\lambda 3}$) were calculated as $\ln 2/\lambda_n$; serum concentration at time 0 (C_0) was calculated as the sum of the intercepts. The volume of distribution V_d in the central compartment was calculated as:

$$V_d = Dose/C_0$$

The area under the serum concentration-time curve ($AUC_{(0\_\infty)}$) and the area under the first moment curve ($AUMC_{(0\_\infty)}$) were calculated by the trapezoidal method with extrapolation to infinity as follows:

$$AUC_{(tlast-\infty)} = C_{last}/\lambda_2$$
$$AUMC_{(tlast-\infty)} = tC_{last}/\lambda_2 + C_{last}/\lambda_2^2$$

where t_{last} is the last time with measurable concentrations (C_{last}) and λ_2 is the rate constant for the elimination phase. Mean residence time (MRT), body clearance (Cl_B) and volume of distribution at steady state (V_{dss}) were determined from the following equations (Gibaldi and Perrier, 1982):

$$MRT = AUMC/AUC$$
$$Cl_B = Dose/AUC$$
$$V_{dss} = Cl * MRT$$
$$V_z = Dose/\lambda_z * AUC$$

Statistical analysis

Pharmacokinetic parameters obtained in males and females were compared to determinate any significant differences using Instat 3.4 (GraphPad Software). The analysis was performed by unpaired t test with Welch correction. Differences with $P< 0.05$ were considered significant.

Pharmacokinetic parameters were reported as means (SD); harmonic means with pseudo-standard deviations were calculated for half lives using a jack-knife technique (Lam et al., 1985).

Means and standard deviations (SD) of intraoperative variables and pain scores were calculated for male and female, separately. The t-test and the Mann–Whitney rank sum test were used to estimate the significance of differences, with $P < 0.05$ considered significant. The analyses were carried out with the GLM-SigmaStat 2.03 software.

Results

Efficacy evaluation

No adverse effects were observed during and after KET administration.

Mean age, body weight, duration of surgery, selected surgical variables, and subjective pain scores are shown in Table 1.

Table 1. Mean (±SD) values of general characteristics, selected surgical variables, and subjective pain score in 15 dogs undergoing surgical gonadectomy.

	Males (n. 5)	**Females** (n. 10)	**Mean** (n. 15)
Age (years)	1.93±1.44	3.45 ± 2.47	2.9 ± 2.2
Body weight (kg)	21.22 ± 7.93	19.45 ± 9.39	20 ± 8.7
Surgery time (min)	27 ± 8.37	66.5±14.73	53.3 ± 23.04
Time from ketorolac injection to start of surgery (min)	20	20	20
Heart rate (per min)	107.60 ± 18.38	106.60 ± 13.61	106.77 ± 15.18
Respiration rate (per min)	10.40 ± 4.16	10.90 ± 4.25	11.63 ± 5.47
MAC % of Iso			1.69±0.11
End tidal CO_2 (mmHg)	40 ± 5	40 ± 5	40 ± 5
Oxyhaemoglobin saturation (%)	>98	>98	>98
End tidal isoflurane (%)	1.48 ± 0.10	1.57 ± 0.12	1.52± 0.30
Subjective pain score	4.40 ± 1.03	3.81 ± 0.56	4 ± 0.57

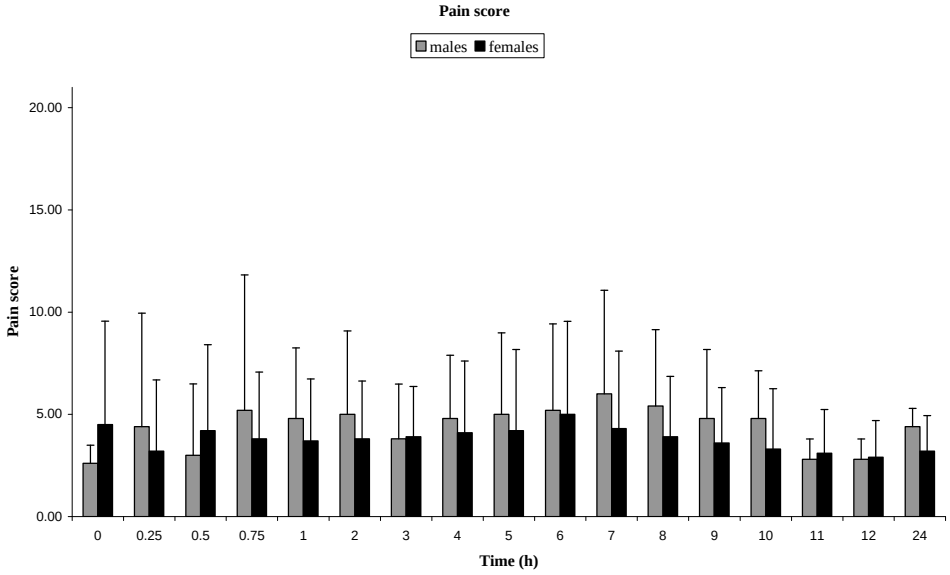
The mean duration of surgery was 27 ± 8.37 minutes for males and 66.5 ± 14.37 minutes for females.

No differences in the physiologic parameters between males and females were recorded during surgery. The mean heart rate was 106.77±15.18 beat/min, the respiratory rate was 11.63±5.47 beat/min and Et-Iso was 1.52 (± 0.22) %. The MAC % of Iso was 1.69±0.11.

Further, there were no significant differences between males and females during pain assessment. The mean values obtained for all the time of observation and for all the parameters considered were 4.40 (± 1.03) for males and 3.81 (± 0.56) for females. However, from Figure 1 the highest values of 6 for males and 5 for females were at 7 and 6 h from KET administration, respectively.

In no case rescue analgesia was necessary because the pain score did not reach the limit values of 9.

Figure 1. Total score of postoperative pain evaluations in the males (M = 5) and females (F = 10) assessed at various times after extubation using the subjective scale pain scoring system method modified by Smith et al. (2004).



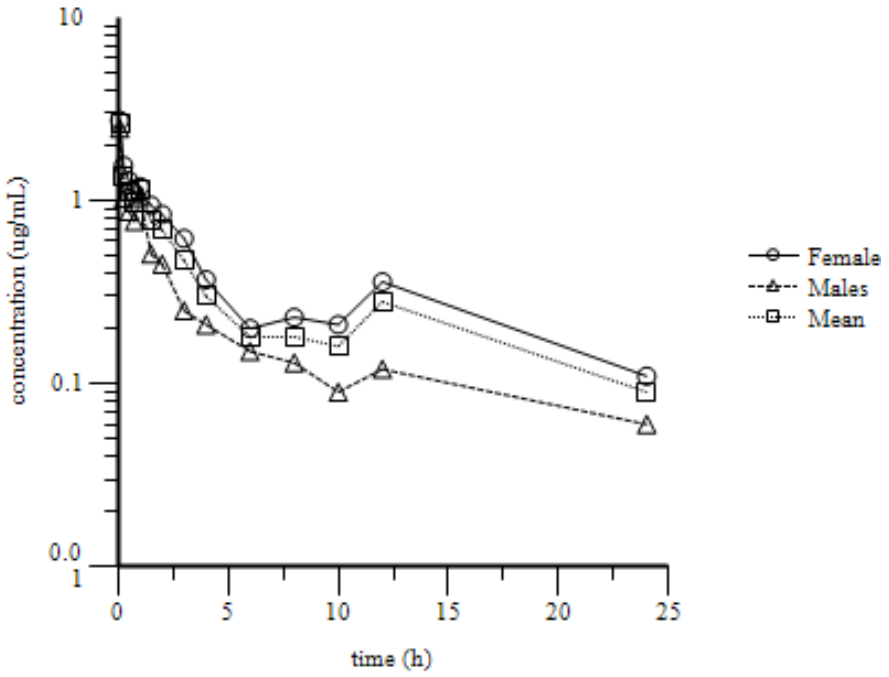
High performance liquid chromatography

The HPLC method was subject to intra-laboratory validation and found to be specific, linear in the range 0.01-10 μg/mL (r^2 = 0.999), precise (4.89-5.50 %) and accurate (3.19-7.50 %). The limit of quantification (LOQ) was 0.01 μg/mL whereas the limit of detection (LOD) was 0.000141 μg/mL. The mean recovery from serum samples was 82.4%.

The percentage of protein binding in the range of concentrations considered was 98.9 % (± 0.13).

Mean serum concentrations of KET, together with mean profiles of drug in males and females, are shown in the Figure 2.

Figure 2. Mean serum concentration of all dogs (dotted line, square), males (dotted line, triangles) and females (continuous line, circles) after IV administration of KET (0.5 mg/kg).



A large variability characterised KET serum concentrations in dogs. In some animals a small increase in the serum concentrations was observed between 1-2 h and between 8-12 h, that was also reflected in the mean value. The mean serum concentration at the first sampling time was 2.65 (± 1.02) μg/mL .After a rapid decrease, in 6 out of 10 subjects an increase of amounts was recorded, *i.e.* from 0.97 (± 0.38) μg/mL at 45 min to 1.13 (± 0.54) μg/mL at 1 h, and a subsequent decrease was observed at 2 h where a mean value of 0.69 (± 0.28) was recorded. The increment noticed between 8-12 h was from 0.18±0.16 μg/mL at 8 h to 0.28±0.21 μg/mL at 12 h in 3 out 10 subjects. KET was still present in all dogs 24 h after administration with a mean concentration of 0.09 (± 0.11) μg/mL.

Pharmacokinetic analysis

The mean pharmacokinetic parameters are reported in Table 2. Although the results were quite variable among subjects, no significant differences were observed between sexes and thus mean values summarise both. Results from 3 out of 10 subjects were best fitted by a three compartmental model, whereas the other results by a two compartmental model. Due to the high variability of the dogs enrolled in this study, the non-compartmental approach was selected to best represent KET profile and thus these parameters will be discussed.

Table 2. Pharmacokinetics parameters of KET after IV administration of 0.5 mg/kg.

Parameter	Unit	Tri-compartmental (n=3)	Bi-compartmental (n=7)	NCA (n=10)
C_{max}		-	-	2.48±1.10
C_0	µg/mL	21.38 ± 8.31	2.67 ± 1.73	
$t_{½\lambda1}$	h	0.022±0.004§	0.21 ± 1.11§	-
$t_{½\lambda2}$	h	1.246±0.52§	12.61 ± 2.25§	10.95±7.06§
$t_{½\lambda3}$	h	38.55±37.95§	-	-
Vd	mL/kg	25.68 ± 9.14	241.70 ± 97.01	-
Vz	mL/kg	-	-	1512.25±799.13
Vdss	mL/kg	4467.51 ± 5312.53	1380.14 ± 969.32	1030.09±620.50
Cl_b	mL/h/kg	56.07 ± 19.32	103.52 ± 101.75	92.66±84.49
$AUC_{(0\rightarrow\infty)}$	h*µg/mL	9.55 ± 2.74	10.92 ± 8.04	9.24±7.16
$AUMC_{(0\rightarrow\infty)}$	h*h*µg/mL	1027.77 ± 1364.17	551.67 ± 743.35	234.73±308.40
MRT	h	95.04 ± 121.15	31.39 ± 36.57	17.54±14.28

C_{max}= maximum serum concentration; C_0= serum concentration at time 0; $t_{½\lambda1}$= distribution half life; $t_{½\lambda2}$= elimination half life; $t_{½\lambda3}$= elimination half life ; V_d= volume of distribution; V_z= volume of distribution based on terminal phase; V_{dss}= volume of distribution at steady state; Cl_b= body clearance; $AUC_{(0\rightarrow\infty)}$= area under serum concentration time curve; $AUMC_{(0\rightarrow\infty)}$= area under moment curve; MRT= mean residence time. § = harmonic mean ± pseudo

The maximum serum concentration (C_{max}) was 2.48 (± 1.10) µg/mL, the elimination ($t_{½\ \lambda2}$) half-life was 10.95 (± 7.06) h. The body clearance (Cl_b) was 92.66 (± 84.49) mL/h/kg, the volume of distribution based on the terminal phase (V_z) was 1512.25 (± 799.13) mL/kg, the volume of distribution at steady state (V_{dss}) was 1030.09 (± 620.50) mL/kg and the area under the plasma concentration time curve ($AUC_{0-\infty}$) was 9.24 (± 7.16) h* µg/mL.

Discussion

KET is a potent anti-inflammatory and analgesic drug currently authorised only in humans. During previous studies in animals, the doses ranged from 0.5 mg/kg in dogs (Mathews et al 1996; Pasloske et al, 1999) to 3.2 mg/kg in the rat (Granados-Sotos et al, 1995).

The dose of KET used in the current study (0.5 mg/kg, IV) was extrapolated from literature data. Mathews et al. (1996) observed that with a dosing schedule of 0.5 mg/kg IM it was possible to obtain pain control during post operative period in dogs, and also to minimize the potential gastrointestinal or renal lesions.

Considering the MAC % of Iso detected in the course of anaesthesia KET should not possess intraoperative analgesic effect. In fact, the MAC % of Iso is considered as an intra-operative parameter to evaluate pain degree during surgery and in dogs anaesthetised by mask and without premedication the MAC% of Iso is 1.30% (±0.10) (Steffey et al., 1994).

All dogs in the present study were pre-medicated with acepromazine that is expected to diminish MAC % of Iso, due to its sedative and anxiolytic effects (Webb and O'Brien, 1998). However, despite the pre-medication, in this study the MAC % of Iso (1.69 % ± 0.11) was about 30 % higher than what reported in literature (Steffey et al, 1994). This increment has been observed notwithstanding the administration 20 min before surgery of KET, that as analgesic drug is expected to decrease the % of MAC Iso.

However, this supposition is not supported by the other intra-operative parameters evaluated. In fact the heart and respiratory rates were constant during all the time of surgery, remaining in the acceptable range of 106.77 ± 15.18 beats/min and 11.63 ± 5.47 beats/min, respectively.

The evaluation of pain degree after surgery shows that all dogs had low levels of pain, as the mean score was under the value of 9 requested for the administration of rescue analgesia, in either males and females. The highest pain score for females and males were recorded at 6 and 7 h, respectively, and they were related with mean KET concentrations of 0.20 (± 0.16) μg/mL for females and 0.15 (± 0.07) μg/mL for males. The KET concentration associated to an analgesic activity in dog is unknown. However, the range between 0.1-0.3 μg/mL is considered to be the EC_{50} value for the KET analgesic effect in humans (Benet et al., 1996). Mean KET serum concentrations remained higher than 0.1 μg/mL up to 12 h from the administration. Although the aim of this study was not to identify an efficacious serum concentration of KET in dog, it is possible to suppose that the analgesic activity in the post-operative period, considering the highest pain score recorded, was maintained at least until 6-7 h after administration, *i.e.* for about 4-5 h after the end of the surgery. KET should be thus administered at least each 8 h, as already stated by Mathews et al. (1996) and Pasloske et al. (1999). In order to maintain the analgesic efficacy after first administration, if necessary, we believe that KET might be re-administered after 6 h. However, a repeated administration study would be advocated to better define the appropriate dosage scheme for KET in the dog.

Soon after administration KET serum concentrations were decreasing as expected, but in few subjects an increase of drug amounts at about 1-2 h after administration was recorded. A similar profile was also observed in rats (Rivera and Espinosa, 2003) and in goats (Nagilla et al., 2009). The reason of this increase of serum concentrations was not further investigated in our study, but could be attributed to an entero-hepatic recycle of drug. In fact Rivera and Espinosa (2003) reported that the administration of KET in rats with cirrhosis, induced by bile duct ligation, did not produce this increase of concentrations, both after IV and oral administration.

Ketorolac was characterised by rapid distribution and quite long elimination. The kinetic behaviour of KET is usually described by a two- or

three-compartment models. Initially, these models have been carried out to calculate the kinetic parameters of KET in our dogs. However, the results obtained did not appear very reliable homogeneous, probably due to the high variability concerning sex, age and metabolic activity of the subjects included in the study. As a consequence, we decided to discuss the non-compartmental analysis and to compare this results with those of the unique other study about KET in dog (Pasloske et al., 1999). Both studies had the same dose, route of administration and product, whereas they differed because our dogs were undergoing surgery and received acepromazine and atropine as pre-medication. It has to be stressed that the presence of other drugs could have influenced the metabolism and the excretion of KET, thus probably explaining the different elimination half-life ($t_{½\,\lambda 2}$), that indeed it was longer (10.95 vs 4.55 h) in our dogs undergoing surgery than in those of Pasloske et al. (1999).

The high KET binding to plasma proteins (98.9%) justified the limited volume of distribution (Vss, 1030.09±620.50mL/kg), as known with NSAIDs (Fonda, 2009).

No adverse effects were observed after IV administration of KET at the dose of 0.5 mg/kg. The lack of undesirable effects in the post-surgical period was verified through a follow up of 30 days by the owners of the dogs, thus supporting the observations of Mathews et al. (1996), that evaluated the gastrointestinal, renal and hepatic effects after KET administration.

Conclusion

The results indicated that KET possess good post-operative analgesic effects in dogs undergoing moderately painful surgery. The drug might be effective until about 6 h after administration, thus a repeated dose study could be carried out in order to better investigate the kinetics and the analgesic efficacy of a multidose therapeutic protocol.

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CHAPTER 6

Pharmacokinetics and efficacy of ketorolac in cats undergoing surgery

In cat pain is under treated for several reasons. Before we can say we have treated pain in cats, we must be able to recognise it and this presents one of the greatest challenges in pain management of cats. Pain is difficult to recognise in this species as they do not demonstrate pain overtly; indicators of pain may be subtle and easily missed even by diligent observers (Lamont, 2002; Lascelles and Waterman, 1997).

Further reasons for under treatment of pain in feline patients are the limited number of analgesics with market authorisation for cats and the fear of side effects. Cats continue to have an undeserved reputation for becoming maniacal and excited when given opioids. This stems largely from historical reports where excessive doses (20-40 times an adequate clinical dose) were given (Fertziger et al., 1974; Watts et al., 1973). With appropriate use, opioids can provide excellent analgesia in cats. The non-steroidal anti-inflammatory drugs (NSAIDs) have also been withheld due to fear of toxicity. NSAIDs should be used with caution in cats because they have a very low capacity for hepatic glucuronidation, which is the major route of metabolism and excretion for this category of drugs.

Compared to other mammals, cats have a very low capacity for hepatic glucuronidation of exogenously administered drugs. Recently, a molecular genetic basis for this deficiency has been identified (Court and Greenblatt, 1997a,b, 2000). Domestic cats have fewer hepatic UDP-glucuroninosyl-transferase (UGT) isoforms, and novel cloning techniques have identified mutations of UGT and pseudogenes. Cats may lack these metabolic pathways because of their carnivorous diet and lack of exposure to plants containing phytoalexins. These metabolic differences can lead to toxic side effects if doses and dosing intervals are not adjusted.

Alternatively, if the parent compound must be metabolised to an active component via this pathway, the drug may be ineffective. Deficient glucuronidation pathways explain the cat's susceptibility to the toxic side effects of phenolic drugs such as paracetamol (acetaminophen) and long half lives of other drugs such as carprofen and acetylsalicylic acid. Cats produce very small amounts of the active metabolite morphine-6-glucuronide (M-6-G) which contributes to the overall analgesic profile of morphine; this may explain because morphine seems less effective in cats compared to other species (Taylor et al., 2001).

However, there is now considerable experience with NSAIDs in cats, particularly the newer compounds, and with appropriate doses and dosing intervals, NSAIDs can be used safely and effectively in this species. Cats require analgesics under the same circumstances as any other species, particularly to treat acute traumatic and peri-operative pain. There are obvious benefits to the cat's

welfare in providing pain relief. In addition, severe pain causes marked physiological effects, and there is substantial evidence supporting the benefits of good pain relief at the time of surgery or trauma in humans (Capdevila et al., 1999; Holte and Kehlet, 2002). The stress response and need for tissue repair after surgery or trauma increases the patient's energy requirements, and if this is not met by an increase in caloric intake, severe weight loss with a negative nitrogen balance develops.

Although objective outcome data are not yet available for cats, the effect of pain on a cat's attitude, and in particular its willingness to eat, are well recognised in clinical practice. Pain relief and positive energy balance are also required for a fully functional immune system, essential for healing in the face of any infection.

Observation of behaviour is undoubtedly the best means of assessing the degree of pain experienced by a cat (Lascelles and Waterman, 1997). Cats in acute traumatic or postoperative pain are usually depressed, immobile and silent. They may appear tense and distanced from their environment and do not respond to petting or attention and may often try to hide. Some cats become manic and aggressive, growl and hiss and roll around their cage. Cats do not like bandages so the observer must differentiate between pain and the dislike of restrictive dressings. Levy et al. (1999) reported that bandages alone caused a 200% increase in urine cortisol, suggesting that cats find this stressful. One important step in pain evaluation is to manipulate the affected area to confirm the presence, or absence of pain.

A wide range of analgesic drugs is utilized to control peri- and post-operative pain such as opioids, local anaesthetics, α_2-agonist and non-steroidal anti-inflammatory drugs (NSAIDs). The use of NSAIDs in veterinary medicine is very common especially for the treatment of musculoskeletal and abdominal pain (Nagilla et al., 2009), thanks to their activity on central sensitization in pathological pain besides the peripheral activity in inflammatory status (Burian and Geisslinger, 2005).

Ketorolac (KET), an heteroaryl acetic acid derivative, is a NSAID approved for use in humans that possesses potent anti-inflammatory, analgesic and antipyretic activities (Sinha et al, 2009). It is commercially available as a mixture of racemate of tromethamine salt, where the S(-)-enantiomer is more biologically active than R(+)-enantiomer (Gillis et al., 1997). Its properties depend on prostaglandin synthetase inhibitory activity and its effectiveness is comparable to morphine, but without troublesome side effects as constipation

and respiratory depression (Anthony et al., 2002). In humans it is used to control the symptomatic relief of moderate to severe postoperative pain, including that associated with abdominal, gynecologic, oral, orthopedic or urologic surgery (Sinha et al, 2009). The KET parenteral administration is advisable for a maximum of 5 days to limit its side effects, such as gastrointestinal, haematological, renal and neurological reactions (Martindale, 2005).

Due to the favourable and potent anti-inflammatory and analgesic activities, KET could represent a useful tool to control acute pain also in animals, such as in the post-operative period. However, the drug is not currently approved for use in veterinary patients. The aim of this study was to determine the pharmacokinetics of KET after a single dose (0.5 mg/kg, IV) and to evaluate its analgesic efficacy in the treatment of post-surgical pain in cats.

Materials and methods

Animals

Sixteen cats (5 males and 11 females), all European breed, from 6 months to 6 years, weighing between 2.8-5 kg, were included in the study. All animals were admitted to the Department of the Clinical Veterinary Sciences of University of Milan for routine surgery and were judged to be healthy (ASA status I) on the basis of physical examination and results of routine blood tests. The study was approved by the Ethical Committee of University and all animals were enrolled after written consent by the owner.

Pre-surgical, surgical and post-surgical procedures

The same anaesthetic protocol was administered in all cats: pre-medication by atropine sulphate (0.03 mg/kg, IM, Atropina solfato, ATI) and acepromazine (0.05 mg/kg, IM; Prequillan, Fatro) and 20 min after pre-medication, all animals were inducted with halothane using an anaesthetic chamber. The anaesthesia was maintained by isoflurane (Isoba, Schering-Plough) in 100% oxygen in spontaneous ventilation.

KET (Toradol, Recordati) was administered as IV bolus at the dose of 0.5 mg/kg after intubation and 20 min prior surgery.

The cats underwent ovariectomy or orchiectomy according to standard surgical procedures. All the surgical procedures were performed by the same surgeon.

During surgery, heart rate, electrocardiogram (lead II), respiration rate, oxyhaemoglobin saturation, end tidal carbon dioxide (Et-CO_2), end tidal

isoflurane (Et-Iso) concentrations and minimal alveolar concentration (MAC%) of isoflurane were recorded every 5 min with Goldway monitor (UT 4000 Fpro).

To evaluate postoperative pain the subjective scores were assessed by a trained observer using a method modified by Smith *et al.* (2004). This scale provides the assignment of a score for each parameter considered (comfort, movement, appearance, unprovoked behaviour, interactive behaviour and vocalization), with a total result ranging from 0 to 24. Pain was assessed starting from extubation (t = 0) at 15, 30, 45, 60 min, then each hour up to 12 h and at 24 hours. Buprenorphine (10 μg/kg IM) was administered if the pain score was 9 or above.

Samples collection and KET extraction

In 9 cats (2 males and 7 females) blood samples were collected at t0 (before KET administration, after induction) 5, 15, 30, 45, 60, 90 min and at 2, 3, 4, 6, 8, 10 and 24 hours after administration of KET. Each sample was centrifuged at 1500 g for 10 min soon after collection and the serum was stored at -20°C until analysis.

The drug quantification was performed using HPLC analytical methods as reported elsewhere (Gallo et al., 2010)

Pharmacokinetic analysis

Pharmacokinetic parameters were deduced from serum concentration-time data using the WinNonLin 5.2.1 Prof software (Pharsight Corporation, USA) which allows compartmental and non-compartmental analyses of the experimental data. Minimum information criterion estimates (MAICE; Yamaoka et al., 1978) were used to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value.

The disposition of KET in cats following IV administration in this study was described by a two compartments model described by the following equation:

$$C_{(t)} = Y_1^{(-\lambda 1t)} + Y_2^{(-\lambda 2t)}$$

where $C_{(t)}$ (μg/mL) is serum drug concentration at time *t*; Y_1 and Y_2 are serum concentrations extrapolated to time zero of the drug distribution and elimination phases; λ_1 and λ_2 are the slopes of the distribution and elimination phases of the drug, respectively. The distribution half-life ($t_{1/2\lambda 1}$) and terminal half-lives ($t_{1/2\lambda 2}$) were calculated as $\ln 2/\lambda_n$; serum concentration at time 0 (C_0)

was calculated as the sum of the intercepts. The volume of distribution V_d in the central compartment was calculated as:

$$V_d = Dose/C_0$$

The area under the serum concentration-time curve ($AUC_{(0\_\infty)}$) and area under the first moment curve ($AUMC_{(0\_\infty)}$) were calculated by the trapezoidal method with extrapolation to infinity as follows:

$$AUC_{(tlast-\infty)} = C_{last}/\lambda_2$$
$$AUMC_{(tlast-\infty)} = tC_{last}/\lambda_2 + C_{last}/\lambda_2^2$$

where t_{last} is the last time with measurable concentrations (C_{last}) and λ_2 is the rate constant for the elimination phase. Mean residence time (MRT), body clearance (Cl_B) and volume of distribution at steady state (V_{dss}) were determined from the following equations (Gibaldi and Perrier, 1982):

$$MRT = AUMC/AUC$$
$$Cl_B = Dose/AUC$$
$$V_{dss} = Cl * MRT$$

Statistical analysis

Pain scores were calculated for male and female, separately. The repeated measures ANOVA with Bonferroni post-test were used to estimate the significance of differences, with $P < 0.05$ considered significant. The analyses were carried out with the GraphPad InStat 3.4 software. Pharmacokinetic parameters were reported as means (SD); harmonic means with pseudo-standard deviations were calculated for half lives using a jack-knife technique (Lam et al., 1985).

Results

Efficacy evaluation

No adverse effects were observed during and after KET administration.

Mean age, body weight, duration of surgery, selected surgical variables and subjective pain score are shown in Table 1.

Table 1. Mean (± s.d.) values of general characteristics, selected surgical variables and subjective pain score of cats undergoing surgery.

	Mean (n. 16)
Age (years)	1.7 ± 1.6
Body weight (kg)	3.3 ± 0.8
Surgery time (min)	20.6 ± 10
Time from ketorolac injection to start of surgery (min)	20
Heart rate (per min)	130 ± 2.80
Respiration rate (per min)	28.12 ± 2.34
MAC % of Iso	1.67 ± 0.08
End tidal CO_2 (mmHg)	40 ± 5
Oxyhaemoglobin saturation (%)	>98
End tidal isoflurane (%)	1.64± 0.22
Subjective pain score males	3.96 ± 1.25
Subjective pain score females	4.81 ± 0.81

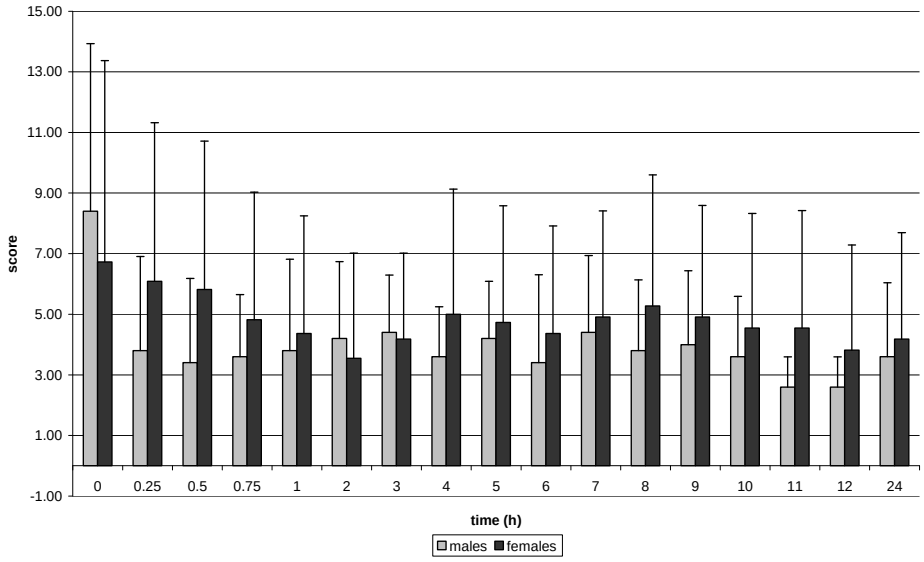
The mean duration of surgery was quite variabile due to the different surgical procedure in males and females.

No differences in the physiologic parameters between males and females were recorded during surgery. The mean heart rate was 130±2.80 beat/min, the respiratory rate was 28.12±2.34 beat/min and Et-Iso was 1.64±0.22 %. The MAC % of Iso was 1.67±0.08.

Significant ($P<0.0001$) differences resulted between males and females in all parameters investigated during the post-operative pain assessment. The mean value obtained for all the time of observation and for all parameters considered was 3.96 (± 1.25) for males and 4.81 (± 0.81) for females. From Figure 1 it is possible to observe that the highest value recorded was at the first observation.

In no case rescue analgesia was necessary because the pain score did not reach the limit values of 9.

Figure 1. Mean pain score in males and females for all parameters considered at different time points.

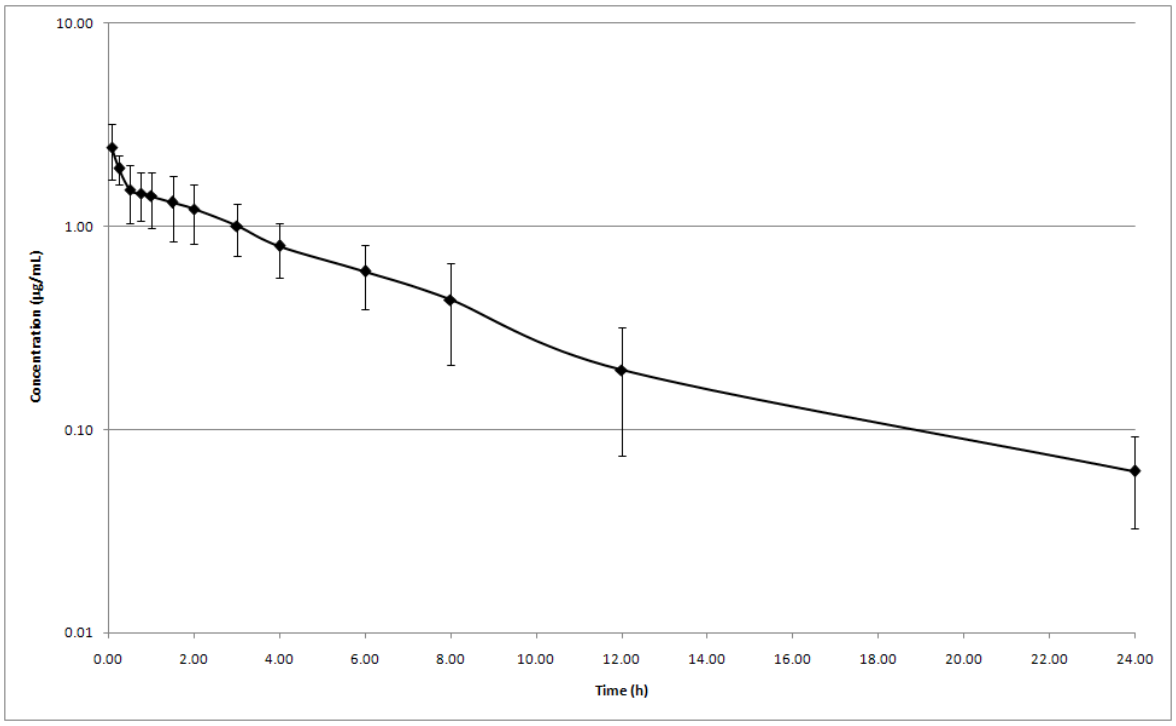


High performance liquid chromatography

The HPLC method, validated intra-laboratory, was specific, linear in the range 0.01-10 μg/mL (r = 0.99), precise (4.80-5.50 %) and accurate (3.20-7.50 %). The limit of quantification (LOQ) was 0.01 μg/mL whereas the limit of detection (LOD) was 0.000141 μg/mL. The mean recovery from serum samples was 82%.

Mean serum concentration of KET, together with mean profile of drug in males and females, are shown in the Figure 2.

Figure 2. Mean serum concentration of all cats after IV administration of KET (0.5 mg/kg).



The mean serum concentration at the first sampling time was 2.44 (± 0.75) µg/mL. There was a rapid decreased in serum concentration and at the third sampling (30 min) mean value was 1.49 (± 0.45) µg/mL. Then concentrations decreased linearly and KET was still present in 8 out of 9 cats 24 h after administration with a mean concentration of 0.06 (± 0.07) µg/mL.

In 7 out of 9 subjects an increase of amounts was recorded between 0.5 h and 3 h. However, this trend was not reflected in mean values, probably because this secondary peaks were observed at different sampling times. It is possible to observe this trend in cats 1 and 2 at the forth sampling with concentration of 1.42 and 1.50 µg/mL, respectively. In cats 3 and 9 this was recorded after 1 hour from administration of KET with a concentration of 1.14 and 2.08 µg/mL. In cats 5, 6 and 7 the increase was observed at different times of sampling (3, 2 and 1,5 h after KET administration) with serum concentrations of 1.36, 0.34 and 1.53 µg/mL, respectively. Cats 4 and 8 did not show an increase in their serum concentration.

Pharmacokinetic analysis

Results from all subjects were best fitted by a bi-compartmental model. The mean pharmacokinetic parameters are reported in Table 2.

Table 2. Pharmacokinetic parameters (mean ± s.d.) of Ketorolac after IV administration in 9 cats at the dose of 0.5 mg/kg

Parameter	Unit	Mean ± s.d. (n=9)
C_0	μg/mL	3.13 ± 1.12
$t_{½\lambda1}$	h	0.54 ± 1.11
$t_{½\lambda2}$	h	4.14 ± 1. 18§
V_d	mL/kg	179.87 ± 66.11
V_{dss}	mL/kg	327.62 ± 121.62
Cl_b	mL/h/kg	56.81 ± 35.12
$AUC_{(0\rightarrow\infty)}$	h*μg/mL	10.67 ±3.89
$AUMC_{(0\rightarrow\infty)}$	h*h*μg/mL	78.95 ±60.41
MRT	h	6.64 ± 3.01

C_0= serum concentration at time 0; $t_{½\lambda1}$= distribution half-life; $t_{½\lambda2}$= elimination half-life; V_d= volume of distribution; V_{ss}= volume of distribution at steady-state; Cl_b= body clearance; $AUC_{(0\rightarrow\infty)}$= area under serum concentration-time curve; $AUMC_{(0\rightarrow\infty)}$= area under moment curve; MRT= mean residence time. § = harmonic mean ± pseudo-deviation standard.

The extrapolated serum concentration at time 0 was 3.13 (± 1.12) μg/mL, the elimination half-life was 4.14 (± 1.18) h. The body clearance was 56.81 (± 35.12) mL/h/kg, the volume of distribution was 179.87 (± 66.11) mL/kg. The area under the plasma concentration time curve ($AUC_{0-\infty}$) was 10.67 (± 3.89) h* μg/mL.

Discussion

The dose of KET used in the current study (0.5 mg/kg, IV) resulted from literature data (Pasloske, 1999), and was also the same used in our study in dogs (Gallo, 2010). In dogs the intramuscular administration of this dose resulted in adequate pain control during the post-surgical period and also minimized the potential gastrointestinal or renal lesions (Mathews et al. 1996). In humans the dosage of KET is quite variable, depending on the age, the route of administration and the pain degree (Gillis and Brogden, 1997). In fact, as a general rule, KET should be administered with the lowest dosage providing adequate analgesia and depending on the patients response. Therefore, the dosage of KET in this study was intentionally low, considering the general indications for the use of NSAIDs in cats and also that gonadectomy should be a moderately painful surgery,

The MAC % of Iso is considered as an indirect intra-operative parameter to evaluate pain degree during surgery. The literature data reported that in cats anaesthetised by mask and without premedication the MAC% of Iso is 1.61% (Drummond et al., 1983). In this study the MAC % of Iso detected during anaesthesia (1.67 % ± 0.08) was about 3.73 % and so higher than what reported

in literature (Drummond et al., 1983). This increment has been observed notwithstanding the administration 20 min before surgery of KET, that as analgesic drug is expected to decrease the % of MAC Iso. Thus, KET should not possess intraoperative analgesic effect.

However, the heart and respiratory rates were in the acceptable range (130 ± 2.80 beats/min and 28.12 ± 2.34 beats/min, respectively) indicating a stable anaesthetic plane and the lack of pain in all animals, thus not completely confirming the above supposition.

Conversely, the analgesic effect of KET was observed during the post-surgical period. The evaluation of pain degree after surgery shows that all cats had low levels of pain, as the mean score was under the value of 9 requested for the administration of rescue analgesia. The highest pain score (6.73 and 8.40 for females and males, respectively) was recorded at time 0, corresponding to the extubation time, probably due to the excitatory phase after awakening typical of this species. Mean KET concentration at the extubation time (calculated to be about 1 hour after administration) was 1.41 (± 0.42) μg/mL, KET concentration associated to the analgesic activity in this species is currently unknown. In humans the EC_{50} value associated to the analgesic activity is reported to be 0.1-0.3 μg/mL (Benet et al., 1996). In this study mean KET serum concentration remained higher than 0.1 μg/mL up to 10 h from the administration (0.26 ± 0.16 μg/mL). Therefore, it is possible to affirm that KET in cats could be efficacious for a long time during recovery. Moreover, additional positive anti-inflammatory effects, as oedema and reddening reduction, were observed in all animals, thus contributing to the overall beneficial properties of KET.

The pharmacokinetic analysis was performed by a two-compartments model in 9 out of 16 animals. The drug was characterised by rapid distribution and elimination. The volume of distribution was quite low, as expected for the NSAIDs. The clearance of KET in cats was quite low (0.94 ml/min/kg). This remark was already observed in dogs undergoing surgery (Gallo 2010), even though the body clearance we reported was slightly higher (1.7 ml/min/kg). The result in cats could be explained by the peculiar characteristics of this species and by the concomitant administration of other drugs included in the anaesthetic protocol, that could interfere with KET metabolism and elimination.

Conclusion

The peri-operative administration of KET in cats undergoing gonadectomy did not cause adverse effects, induced adequate analgesia and anti-inflammatory effects during the post-operative period. The dosage adopted (0.5 mg/kg IV) was likely suitable to provide sufficient analgesia during the entire recovery period. Despite the well-known difficulties in NSAIDs use in cats, the

KET low dosage of we investigated could be safely used as analgesic and anti-inflammatory drug in multimodal protocols for moderately painful surgery.

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CHAPTER 7

Pharmacokinetics of ketorolac in horses undergoing orchiectomy

This experimental part has been developed as short communication since it is only a preliminary study.

Veterinary practitioners have recently increased their attention about pain management and the improvement of welfare of their patients. Untreated pain decreases quality of life, prolongs recovery from surgery or illness and, in some cases, could induce anatomical damages that lead to persistent pain, hyperalgesia or allodynia. According to the preemptive analgesia concepts (Corletto, 2007) the administration of analgesic drugs in the pre-operative period can positively influence the analgesic effects during the recovery and the post-operative period. The anti-inflammatory drugs (NSAIDs) are the most used analgesic drugs in equine practice, as recently confirmed by the survey conducted among members of The American Association of Equine Practitioners (Hubbell, 2010). Due to the favourable and potent anti-inflammatory and analgesic activities in humans, ketorolac (KET) could represent a useful tool to control acute pain in animals as well. This drug is not currently approved for the use in veterinary practice. The aim of the study was to determine the pharmacokinetics and the analgesic efficacy of KET after a single dose (0.5 mg/kg, IV) administered in the pre-operative period in horses undergoing gonadectomy.

Five male horses coming from the same breeding were involved in the study. The animals, weighing between 230-370 kg (mean 297.6 ± 46.3) and 2 years old, were admitted to the Veterinary Hospital of University of Milan for orchiectomy. All animals were judged healthy (ASA status I) on the basis of physical examination and results of routine blood tests. The study was approved by the Ethical Committee and the owner of animals was asked to sign the written consent. During the study all animals were housed in single boxes and food and water were provided *ad libitum*. The animals were acclimated for 2 days before surgery. Food was withdrawn 12 hours before surgery.

The animals were pre-medicated with 0.05 mg/kg of acepromazine maleate and 0.015 mg/kg of detomidine intramuscularly (IM) and the anaesthesia was inducted with ketamine (2.2 mg/kg) and diazepam (0.05 mg/kg). After intubation the animals were maintained with isoflurane in oxygen (100%) in intermittent positive-pressure ventilation (IPPV) to maintain end-tidal carbon dioxide values between 39 and 42 mmHg. During anaesthesia, variations of isoflurane concentration were performed to maintain an appropriate depth of anaesthesia based on clinical assessment; signs monitored included degree of nystagmus, movement, muscle relaxation, response to surgery, invasive blood pressure (IBP), heart rate (HR). Ketorolac tromethamine (Lixidol 30 mg/ml) was administered IV after the induction and about 10 minutes before the beginning of the surgery at the dose of 0.5 mg/kg b.w.

During surgery IBP, HR electrocardiogram (lead II), oxyhaemoglobin saturation (SpO_2), end tidal carbon dioxide ($EtCO_2$), invasive systolic arterial pressure (SAP), invasive mean arterial pressure (MAP) and invasive diastolic arterial pressure (DAP), were recorded every 5 min (UT4000F Pro monitor, Goldway Inc., USA).

Blood samples for drug quantification were collected in non-heparinized tubes before administration (t0) and at pre-established times until 36 hours after administration. Serum was obtained by centrifugation at 1200 *g* for 10 min and immediately frozen at -20°C pending assay.

Drug quantification was achieved by HPLC with UV-visible detection as described elsewhere (Gallo et al., 2010 a,b) and the analytical method was validated intra-laboratory.

The pharmacokinetic analysis was deduced from serum concentration-time data using Phoenix Win NonLin® 6.1 (Pharsight Corporation, USA) which allows compartmental and non-compartmental analysis of the experimental data. Minimum information criterion estimates (MAICE, Yamaoka 1978) were used to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value. Serum concentrations of KET in horse was fitted by a two compartments model according to the following equation:

$$C_{(t)} = Y_1^{(-\lambda 1 t)} + Y_2^{(-\lambda 2 t)}$$

where $C_{(t)}$ (μg/mL) is serum drug concentration at time *t*; Y_1 and Y_2 are serum concentrations extrapolated to time zero of the drug distribution and elimination phases; λ_1, and λ_2 are the slopes of the distribution and elimination phases of the drug, respectively. Pharmacokinetic parameters are reported as means (± s.d.) in the Table 1. Harmonic means with pseudo-standard deviations were calculated for half lives using a jack-knife technique (Lam 1985).

Table 1. Mean pharmacokinetic parameters after IV Ketorolac tromethamine administration in 5 horses at the dose of 0.5 mg/kg.

Parameter (units)	mean (± s.d.)
AUC (h*μg/mL)	1.75 (±1.03)
$t_{1/2\lambda1}$ (h)	0.06 (± 0.02) [a]
$t_{1/2\lambda2}$ (h)	0.59 (± 0.21) [a]
K_{10} _HL (h)	0.14 (± 0.12) [a]
K_{10} (1/h)	5.37 (± 4.14)
K_{12} (1/h)	4.67 (± 3.65)
K_{21} (1/h)	3.35 (± 2.77)
V_1 (mL/kg)	107.55 (± 78.24)
Cl_B (mL/h/kg)	339.99 (± 120.19)
AUMC (h*h*μg/mL)	0.87 (± 0.28)
MRT (h)	0.59 (± 0.29)
V_{ss} (mL/kg)	218.83 (± 134.26)

$AUC_{(0-\infty)}$ = area under serum concentration-time curve from 0 extrapolated to infinity; $t_{½\lambda1}$ = distribution half-time; $t_{½\lambda2}$ = elimination half-time; K_{10} = the rate at which the drug leaves the system from the central compartment (the elimination rate); K_{12} = the rate at which the drug passes from central to peripheral compartment; K_{21} = the rate at which the drug passes from peripheral to central compartment; K_{10} _HL = the half-life associated with the rate constant K_{10}; V_1 = volume of distribution in central compartment; Cl_B = body clearance; AUMC = area under moment curve; MRT = mean residence time; V_{ss} = volume of distribution at steady state

[a] harmonic mean ± pseudo SD

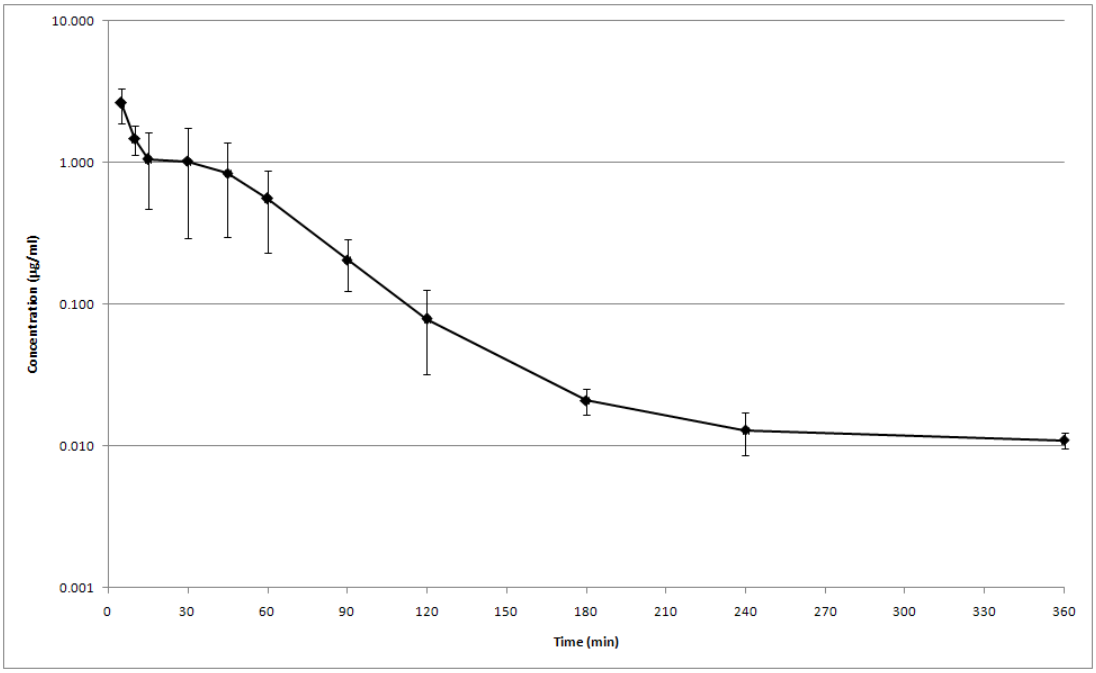
All animals were monitored to assess the pain degree in the postoperative period (yes *vs* no: signs of pain present *vs* absent). The severity of pain was evaluated by a visual analogue scale (VAS) that provide semi-objective scoring method for evaluating pain in horses. The observer was asked to judge the animal status by placing a time-dated mark on a 10 cm line, where 0 was no pain and 10 the maximum pain, at 30 min, 1, 2, 3, 4, 6, 8 and 12 h after the extubation of the animals. For this study "No pain" was considered in the VAS from 0 to 3 cm, "Moderate pain" was considered from 3 to 6 cm and "Worst pain" was considered from 6 to 10 cm. Pain was judged unacceptable if a score ≥ 5 cm was awarded using VAS. The "rescue analgesia" protocol was 0.1 mg/kg of butorphanol IV.

Differences in the individual pain scores at the same observation time were investigated using one-way analysis of variance (ANOVA) (GraphPad Software, Instat 3.4)

The intra-operative physiological parameters monitored were included in the normal range. The mean surgery duration was 33.5 minutes (± 13.17). Drug concentrations were quite low (2.63 ± 0.72 at 5 mins after administration) and rapidly decreased. KET was quantified (Figure 1) with amounts close to the limit of quantification of the method (LOQ = 0.01 μg/ml) from 3 hours after

administration, even though it was still detectable until about 6 hours in the same range of values.

Figure 1. Mean (± s.d.) serum concentration of Ketorolac tromethamine administered IV in 5 horses at the dose of 0.5 mg/kg.

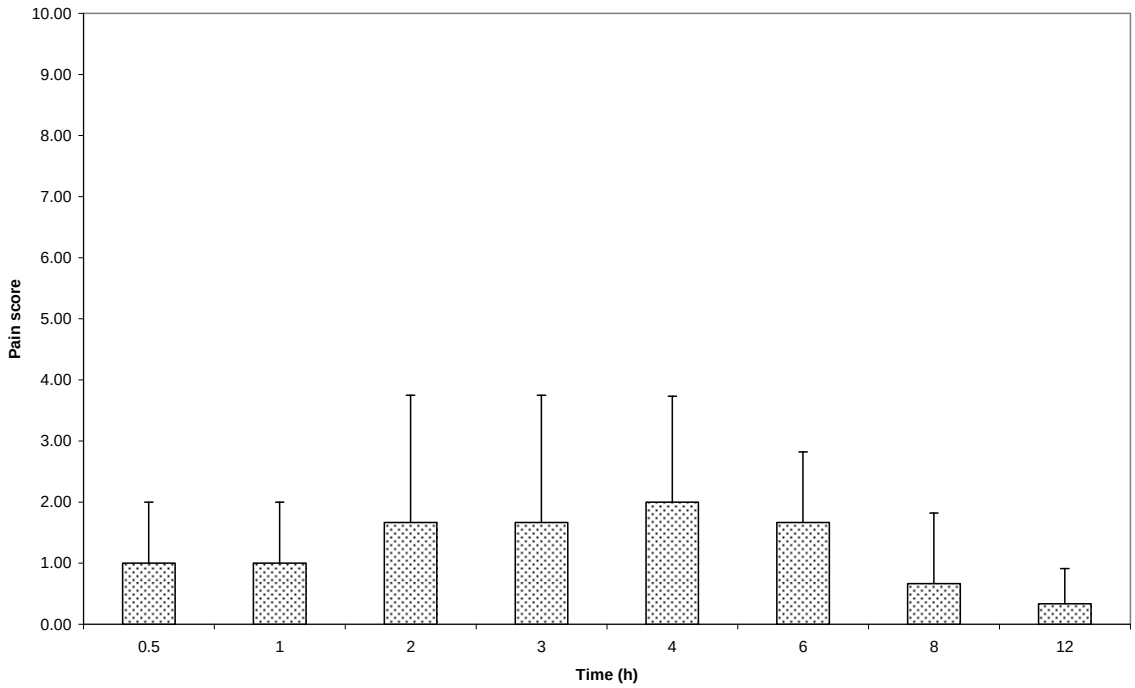


Drug serum profile was highly variable among subjects, particularly during the first part of the curve. In all subjects a secondary peaks were recorded in a range from 15 and 45 min after administration. This remark is quite common after KET administration also in other species (Gallo et al., 2010a,b; Nagilla et al., 2007) and it might indicate that an entero-hepatic re-circulation of the drug occurs in horse. The pharmacokinetic parameters derived by the bi-compartmental analysis are resumed in the Table 1. KET was characterized by a very rapid distribution ($t_{1/2\lambda1}$ = 3.6 mins) and elimination ($t_{1/2\lambda1}$ = 35.4 mins) half-lives. These values are similar to those reported in sheep after IV dose of 2 mg/kg b.w. (Santos 2001), but shorter than in the dogs and cats we investigated with the same dosage (Gallo 2010a – b) and in other species with higher dosage (Mroszczak 1990, Nagilla 2007, Nagilla 2009). In horses drug clearance was low (about 5.6 ml/min/kg), even though higher than in dogs and in cats we investigated with the same dosage (Gallo et al., 2010 a,b). It is difficult to compare our values with those recorded by other authors (Santos 2001, Nagilla 2007, Nagilla 2009), first because dosages and species are different, and second because our animals were undergoing surgery, thus the concomitant presence of

other drugs and the hemodynamic variations during anesthesia could have interfered with the overall metabolism.

According to the preemptive analgesia concepts, KET was administered before the surgery. However, drug concentrations during the recovery time were quite low. When animals achieved the extubation time (mean 15.4 ± 7.7 mins) KET concentration was meanly of about 1 μg/ml, whereas when animals were standing (34.6 ± 9.4 mins from the end of the surgery, *i.e.* about 1.5 hours after drug administration) KET concentrations were about 0.21 ± 0.08 μg/ml. Therefore, during the post-operative period drug concentrations were very low (0.08 ± 0.05 μg/ml at 2 h), and in most of the animals the drug was completely undetectable starting from 4 hours after administration. The effective analgesic concentration of KET in horse is not available. In humans an EC_{50} of 0.1-0.3 μg/ml is reported (Pasloske, 1999). The E_{max} achieved in rats after a dose of 0.3 mg/kg was about 20% and the C_{max} was about 0.28 μg/ml (Granados-Soto et al., 1995). The scores of pain assessment (Figure 2) were low and always under the limit for the rescue analgesia (≥ 5).

Figure 2. Mean pain score (± s.d.) after IV Ketorolac tromethamine administration in 5 horses at the dose of 0.5 mg/kg.



However, a slight increment in the score for few subjects (P = < 0.05) was observed from 2 to 6 hours, when the drug concentrations were already under the analgesic levels reported above. However, since KET is an analgesic

drug for moderate to severe pain, according to the pain scores recorded in all animals and to the fact that rescue analgesia was unnecessary, it is possible to suppose that the anti-inflammatory and analgesic effects were enough for a moderately painful surgery such as the orchiectomy. No adverse effects due to KET administration were reported during the recovery of the animals. Therefore, it is possible to suggest that KET administration at the dose of 0.5 mg/kg b.w. could be an useful tool to control the post-operative pain after moderate painful surgery in horses. However, future studies with higher dosages are suggested in order to prolong the analgesic and anti-inflammatory effects and thus investigate the benefic effects of KET towards more painful surgery.

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CHAPTER 7

Conclusions

Veterinary practitioners have recently increased their attention about pain management and the improvement of welfare of their patients. The attention of clinicians is mainly directed on treatment of acute pain due to trauma or surgery. In the case of surgery the analgesic strategy is the preemptive analgesia, *i.e.* the administration of analgesic drugs before pain stimuli to reduce the development of pain mechanisms. The choice of the analgesic drug depends on pathology, drug safety and efficacy, habit and knowledge of the clinician, practicalness of drug administration by the owner and, not less significant, the economic aspect.

In small animals and equine practice the administration of NSAIDs and opioids represents the most frequently used tool of managing postoperative pain. However, routine use of analgesics is not yet uniform and usually the protocols of treatment are extrapolated from clinical experience in humans.

The studies reported in this thesis aimed to evaluate the analgesic effectiveness and the pharmacokinetics of two substances (tramadol and ketorolac) that are representative of the two main classes of analgesic drugs used in veterinary medicine, *i.e.* opioids and NSAIDs.

The preoperative administration of the opioid tramadol at the dose of 2 mg/kg was carried out in dogs undergoing TPLO in cats (2 mg/kg) and horses (4 mg/kg) undergoing gonadectomy. The drug produced adequate intra- and postoperative analgesia without significant side effects in all species.

The preoperative administration of the NSAID ketorolac was carried out at the dose of (0.5 mg/kg) in dogs, cats and horses undergoing gonadectomy. The administration induced adequate post-operative analgesia without adverse effects in all species, even though an higher dosage in horses should be recommended. Moreover, the anti-inflammatory effects of drugs were observed and contributed to the overall welfare in the post-operative period.

The multimodal analgesia is the simultaneous administration of analgesic drugs belonging to different pharmacological families and characterized by different mechanisms of action to produce synergistic effects and to reduce drugs doses and the incidence of adverse effects.

According to this concept, the combinations of opioids and NSAIDs are commonly used to control postoperative pain. The potential advantage of using combination therapy is that analgesic effects can be maximised while the incidence of adverse effects is minimized. Therefore, using combinations of

medications that offer analgesic synergism should allow a reduction in required dosage and decrease the incidence of adverse effects.

Lòpez-Muňoz et al. (2004) demonstrated that the oral co-administration of tramadol and ketorolac in rats produced an antinociceptive effect greater than that observed after individual treatment. In this study the potentiated antinociceptive effects were not accompanied by increased side effects and the consumption of ketorolac and tramadol was significantly lower when the two drugs were administered together.

Lepri et al. (2006) compared the clinical advantages and disadvantages of patient-controlled-analgesia with continuous infusion with tramadol alone *versus* a combination of tramadol plus ketorolac in the management of post-operative pain after major abdominal surgery in humans. This study demonstrated that ketorolac could be used as an effective and safe adjuvant to tramadol for post-operative analgesia after abdominal surgery.

Based on this preliminary results in laboratory animals and in humans, it could be of interest to investigate the synergic activity of tramadol and ketorolac in veterinary medicine in order to evaluate effectiveness and safety of their co-administration for perioperative analgesia. Due to their characteristics, tramadol might cover the intra-operative period, whereas ketorolac, according to its anti-inflammatory and analgesic activities, might prolong the beneficial effects during the post-operative period. The remarks resulting from this thesis highlight the likely synergic activities of the two drugs that could be useful tools to control also for moderate to severe pain.

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