LETTER TO THE EDITOR

SUBSTANCE P EXPRESSION IN THE GINGIVAL TISSUE AFTER UPPER THIRD MOLAR EXTRACTION: EFFECT OF KETOPROFEN, A PRELIMINARY STUDY

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The aim of this study was to evaluate substance P (SP) levels and the effect of a non-steroidal anti-inflammatory drug (NSAID), ketoprofen, on SP in the pericoronal gingival tissue after extraction of upper third molars. A sample of 20 young non-smoking systemically healthy adults of both sexes, with a healthy upper third molar to extract for orthodontic purposes, was selected. After extraction, a sample of the gingival tissue of the pericoronal region was collected with a sterile scalpel, placed into test tubes and kept frozen at -20°C until the SP determination. SP levels were determined by using a commercially available enzyme immunoassay (ELISA) kit. The subjects were randomly divided into two groups: group 1 received a single dose of ketoprofen 30 minutes prior to the experimental procedure. The subjects of group 2 did not receive any kind of drug administration before extraction. The patients were asked to complete a diary on the postoperative pain. A relevant amount of SP was measured in all the gingival samples. No statistically significant difference could be detected in SP expression between the two groups. In group 1 pain appearance was significantly delayed (6.2±0.13 hours) in comparison with group 2 (3.95±0.2 hours). In this small selected group of subjects and limited study design, preventive administration of ketoprofen did not significantly affect the gingival levels of SP, the clinical recommendation emerging is that of NSAID administration postoperatively but before pain appearance in order to optimize the management of pain of the patient.

To the Editor,

Findings suggest a potential role for substance P (SP) in the gingival tissues, and in particular in the resident fibroblastic cells and a relationship between the neuropeptide release and neurogenic inflammation (1). SP is produced in a subset of capsaicin-sensitive sensory peripheral neuron cell bodies localized in the dorsal root and trigeminal ganglia (2), and plays an essential role in the transmission of noxious stimuli in the spinal cord. The stimulation of capsaicin-sensitive sensory peripheral terminal of the neurons results in the peripheral release of several neuropeptides including SP (3, 4, 5), and evidence supports in particular a role of SP in the development and maintenance of dental pain and inflammation (6). Previous findings have demonstrated how SP

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represents a key neuropeptide in the generation of neurogenic inflammation as in presence of caries (7), occlusal trauma (8), after cavity preparation (9), dentin-bonding agent application (10), after the application of tooth bleaching products (11) and in orthodontic movements (12). Several studies have shown that SP expression is significantly increased in the inflamed pulp, suggesting that SP plays a key role in the pulpal inflammatory process (13).

Non-steroidal anti-inflammatory drugs (NSAIDs) are medications used frequently all over the world. NSAIDs may adequately control postoperative symptoms after different dental procedures such as the removal of third molars. Effective analgesia provides the patient with a better quality of life in the postoperative period, allowing faster recovery and earlier return to their daily activities. Common forms of postoperative pain prevention include the preoperative administration of NSAIDs (14, 15). The degree of nociception of patients seems to be related in part to the concentration of histamine, kinines, and prostaglandins at the site of inflammation. The maximum concentration of prostaglandins in acute tissue injuries occurs simultaneously with the peak of postoperative pain intensity (3 to 4 hours after injury). NSAIDs are capable of limiting peripheral sensitization by reducing prostaglandin synthesis at the site of surgery, however, few studies have evaluated the role of NSAID administration on neurogenic inflammation (13). This study aimed to measure SP expression in the gingival tissue after third molar extraction and to evaluate the effect of preoperative ketoprofen administration, a non-steroidal anti-inflammatory drug (NSAID), on the quantity of SP in this tissue and on the degree of nociception of the patients.

MATERIALS AND METHODS

A sample of 20 (19-30 years old) systemically non-smoking healthy adults of both sexes was selected among the patients of the Department of Orthodontics of the University of Insubria, Varese, Italy. Inclusion criteria comprehended the presence of a healthy upper third molar programmed for extraction for orthodontic purposes. Written informed consent was obtained from each patient. The study was performed according to the recommendations regarding ethical issues in research with human tissues of the University of Insubria of Varese, Italy, and was conducted in accordance with the Declaration of Helsinki. The subjects were randomly divided into two groups: group 1 received a single dose of 80 mg ketoprofen lysine salt (Oki® manufactured by Dompe CPA, Italy) in the form of sachet to dissolve in water, 30 min prior to the experimental procedure. The subjects of group 2 did not receive any kind of drug administration. Prior to extraction the teeth were anaesthetized (1.8 mL of 4% articaine with 1:100.000 epinephrine by infiltration injection); 15 min later a sample of the gingival tissue from the pericoronal region of the extraction site was collected with a sterile scalpel and placed into a test tube and kept at -20°C until the SP expression. The wound was then sutured and the patient was asked to complete a diary on the postoperative pain until the control appointment seven days later, in which time of appearance and recurrence of pain, necessity of use ketoprofen, and efficacy of the drug in controlling pain were analysed. A verbal pain intensity scale was used, where pain intensity was indicated as: no pain, slight, moderate, intense, severe (16). Before surgery, the patients were carefully instructed on the compilation of the diary, and were further interviewed at the control appointment about the modalities of compilation in order to check the effectiveness of pain records. For SP determination, gingival samples were weighed and 0.5 ml of Acetic Acid 0.5N, containing a protease inhibitor cocktail (Roche, Milano, Italy), was added to all samples which were then boiled for 10 min. Sample were homogenized and centrifuged at 10,000 x g for 10 min at 4°C. Supernatants were collected, dried with a Speed Vacuum Concentrator and resuspended in 0.3 ml of assay buffer. SP levels were determined by using a commercially available enzyme immunoassay (ELISA) kit according to the manufacturer's instructions (R&D system, Milano, Italy). Sensitivity of the kit was 15 pg/ml. Non-parametric Mann-Whitney U test was used to analyse differences in pain duration and in SP levels since normality test failed.

RESULTS

A relevant amount of SP was measured in all the gingival samples. SP concentrations, expressed as pg/mg of tissue of each patient are reported in Tables I and II. As reported in Table III, no statistically significant difference was present in SP levels between the two groups. In Tables I and II also appearance of pain after extraction (expressed in hours), intensity of pain (classified by patients according to: no pain; slight; moderate; intense), time of reduction of pain after ketoprofen intake (expressed in minutes) for the patients who felt the necessity to take it, and the duration of pain relief (expressed in hours) are reported. Generally, patients experienced only slight-to-moderate pain, and only one patient described pain as intense.

In group 1, appearance of pain was significantly delayed (6.2±0.13 hours) in comparison to patients who did not receive ketoprofen before the experimental procedure (3.95±0.2 hours). This difference resulted statistically significant ($p < 0.0001$, Mann-Whitney U test), as reported in Table III. Half of the patients in group 1 felt the necessity to take a second ketoprofen dose after tooth extraction, while in group 2 all the patients decided to take the drug at the appearance of pain after the surgical procedure.

DISCUSSION

This study investigated the expression of SP and the effect of ketoprofen administration, a commonly used non-steroidal anti-inflammatory drug (NSAID), on the quantity of SP in the gingival tissue after upper third molar extraction. In the patients of group 1 a single dose of ketoprofen lysine salt ("Oki", Dompe CPA, Italy) was administered 30 minutes prior to extraction while in group 2 no drug was administered before the procedure. No statistically significant difference in SP expression could be detected between the two groups.

Caviedes and coworkers (17) reported an SP concentration of 0.453 pmol per mg of periodontal ligament of intact teeth; SP levels have also been

Table I. *Substance P and pain evaluation after tooth extraction of upper third molars, in patients with ketoprofen administration 30 minutes before the procedure.*

Patients group 1	SP pg/mg gingiva	Appearance of pain (hours after extraction)	Intensity of pain	Onset to pain relief (minutes after second ketoprofen intake)	Duration of pain relief (hours)
1	14.03	7	slight	no drug	
2	147.17	6	moderate	30	> 5
3	39.1	6	moderate	30	> 5
4	18.61	6	slight	no drug	
5	77.72	7	slight	30	> 5
6	42.8	6	slight	no drug	
7	32.87	6	moderate	no drug	
8	15.24	6	moderate	30	4 - 5
9	43.62	6	slight	no drug	
10	21.99	6	moderate	30	3 - 4

Table II. *Substance P and pain evaluation after tooth extraction of upper third molars in patients with no drug administration before the procedure.*

Patients group 2	SP pg/mg gingiva	Appearance of pain (hours after extraction)	Intensity of pain	Onset to pain relief (minutes after ketoprofen intake)	Duration of pain relief (hours)
1	40.99	3	moderate	30	> 5
2	43	4.5	slight	30	> 5
3	36	4	moderate	30	3 - 4
4	89.67	5	moderate	30	4 - 5
5	14.26	5	slight	30	> 5
6	33.80	3	slight	30	3 - 4
7	46.44	4	moderate	30	> 5
8	10.69	3.5	slight	30	> 5
9	19.35	3.5	slight	30	3 - 4
10	52.0	4	intense	30	4 - 5

Table III. *Pericoronal Substance P levels and time to appearance of pain.*

	Group 1 (ketoprofen)	Group 2 (no drug)	Mann-Whitney U test
Gingival SP (pg/mg tissue) (mean±SEM)	45.31±12.81	38.62±7.18	p= 0.9
Appearance of pain (hours) (mean±SEM)	6.2±0.13	3.95±0.23	p< 0.0001

investigated in the pulp and in the gingival crevicular fluid, however, no data is available on the pericoronal gingival tissues. The presence of increased SP levels could open new directions for treating inflammation and pain occurring after oral surgical procedures. In this study, considered preliminary due to the limited number of patients, Ketoprofen did not seem to interfere significantly on the SP expression in the pericoronal tissues after third molar extraction. Considering the efficacy of this molecule in the modulation of pain and inflammation in third molar surgery previously reported (18), this result may appear rather disappointing and could be interpreted as inability of the drug to prevent SP increase during surgical procedures even if the capacity to produce analgesia in the postoperative period is widely reported. These data are in accordance with a previous study evaluating SP levels in the pulp after induced lesion (13).

The data of the present study are in accordance with evidence (14) suggesting that there seem to be no basis for pre-emptive analgesia with various NSAIDs in the third-molar surgical model. No prevention of postoperative pain was here reported, the only modification was a significantly postponed appearance of pain after tooth extraction with preventive ketoprofen administration (6.2±0.13 hours *vs* 3.95±0.2 hours). According to Kaczmarzyk and coworkers (19) ketoprofen was most effective when administered in the postoperative period. Liporaci Junior (20) showed no significant difference using ketoprofen for pre-emptive analgesia in surgical extraction of third molars. According to the data of this preliminary study, 5 patients of 10 of group 1

used the drug also postoperatively, 5 patients of 10 of group 2 took more than one dose of ketoprofen, so pre-emptive administration did not seem to result in reduction of pain and of quantity of painkillers used in the postoperative period.

In conclusion, ketoprofen does not seem to interfere significantly on the SP expression in the pericoronal tissues after third molar extraction. Considering the efficacy of this molecule in the modulation of pain and inflammation in third molar surgery this result could be interpreted as inability of the drug to modulate SP during surgical procedures even if the capacity to produce analgesia in the postoperative period is widely reported. More studies with other molecules and performed on larger samples are necessary to evaluate the effect of NSAIDs on SP expression in the gingival tissues. In this small, selected group of subjects and limited study design the clinical recommendation that seems to emerge is that of NSAID administration postoperatively but before pain appearance in order to optimize the management of the postoperative pain of the patient.

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