Physiologic variations of TLI (trypsin like immunoreactivity) in serum of german shepherd dog and correlation with gastrin levels

E. Cavallone**, M. Gualtieri*, M. Corti*, A. Ciorba***, E.M. Rimoldi*, L. Leonardi*
* Istituto di Clinica chirurgica e radiologia veterinaria, Università degli Studi di Milano, Italy
** Laboratorio di radioisotopi di medicina veterinaria, Università degli Studi di Milano, Italy
*** Dipartimento di scienze biopatologiche, Università degli Studi di Perugia, Italy

Summary

The term trypsin-like immunoreactivity (TLI) defines the fraction of trypsin and trypsinogen which can be determined with immunochemical reactions. The physiological and pathological role of this parameter in the dog is reviewed. The aim of present study was to investigate the physiological variations of TLI in 40 healthy German shepherd dogs, 20 females and 20 males. The animals under study were divided in 5 age groups of 8 animals each (4 females and 4 males) ranging from 3 months to 10 years. A commercial, double-antibody, dog-specific, RIA kit has been employed to determine TLI. The reference range of TLI obtained for the German shepherd dog (5.7-19.3 ng/ml, SD 3.2) was comparable to that reported in literature for other breeds of dogs (5.2-35 ng/ml). No difference in the TLI concentration in relation to sex has been observed (females 12.6 ng/ml, SD 3.2; males 12.3 ng/ml, SD 4.6). Even though still within the normal limits, a higher TLI concentration was observed in the group of subjects aged 3-3.5 months (15.9 ng/ml, SD 3.7).

The TLI activity stabilized in the subsequent age groups from 7 months to 10 years, with no significant variations. The variations, in percentage terms, of TLI serum concentrations have been compared to those observed for gastrin in a previous study of the same authors. This comparison revealed a strict correlation between the variations observed in the different age groups of these hormones, both important in the digestion and absorption of proteins. This study supports the need for critical evaluation of pathological variations of TLI and gastrin in serum of the dog.

Introduction

The term TLI (Trypsin-like immunoreactivity) refers to the fraction of trypsinogen and trypsin present in the bloodstream and recognizable by immunochemical reactions (Braun and others, 1997). Trypsinogen is secreted by exocrine pancreas and, converted into trypsin, enables the digestion of proteins from food. In Figure 1 a simplified description of the mechanisms regulating the synthesis of TLI and the complex links known in literature with the other hormone systems is reported. In particular, it is important to consider the relationship with the regulation of the secretion of gastrin at the vagal level, as this has direct synergistic activity on pancreatic secretion (Agugini and others, 1996; Braun and others, 1997; Faglia, 1997; Ghatei and others, 1997; Inoue and others, 1985; Johnson and others, 1986; Le Drean. and others, 1998; Nustede and others, 1993; Shulkes and Daldwin, 1997; Williams, 1989). TLI is strictly organ-specific and therefore abnormal variations in serum suggest pancreatic damage. Nevertheless, being excreted by the kidney, its serum concentration can rise also in the presence of nephrosis (Archer F.J. and others, 1997; Koop and others, 1980; Geokas and others, 1982; Simpson and other, 1991; Williams and Batt, 1983; Williams and Batt, 1986; Williams and Batt, 1988; Williams, 1987; Williams, 1989; Williams, 1990; Williams, 1994). Therefore, in routine clinical investigations it is necessary to combine TLI determination with parameters of kidney function. In the case of acute pancreatitis its increase in systemic circulation appears earlier than that of amylase and lipase, and decreases faster during the re-
mission of the disease. Important diagnostic and prognostic information deduced from this behaviour, has been discussed elsewhere (Archer, 1997; Braun, 1997; Williams, 1994). In pancreatic failure the TLI levels in serum decrease remarkably, and its determination furnishes a clear picture of the severity of the disease and is useful in monitoring of treatment (Boari and others, 1993; Braun, 1997; Dotta and others, 1985; Williams and Batt, 1983). Requiring just the analysis of a single serum sample, the TLI determination is easier and less time-consuming compared to other biochemical investigations. For example, the determination of faecal chymotrypsin, even though useful and meaningful, requires the analysis of many portions of the same sample (Abate and Massirio, 1985; Boari and others, 1993; Pozza and others, 1989; Williams and Batt, 1988). In any case, to follow the course of the disease and the efficacy of the therapy, serial determinations in the time are necessary.

The aim of present study has been to establish in German shepherd dog the reference range of TLI, and its eventual variations in relation to age and sex. In addition, a comparison with the variations of gastrin, in the same species and at different ages, has been performed.

Materials and methods

Blood samples have been drawn from 40 German shepherd dogs, 20 female and 20 male, of the Perugia Revenue Guard Corps breeding. The samples were taken in the course of routine health checks. The dogs were grouped according to the age in groups of 8 subjects, 4 female and 4 male, each. The age ranges were:

1st group: 3-3.5 months
2nd group: 7-15 months
3rd group: 2-3 years
4th group: 4-6 years
5th group: 7-10 years
All the subjects have been submitted to clinical examination and routine laboratory tests (complete blood count, blood glucose, creatinine and urea). All the subjects had all the laboratory values within normal ranges. The blood samples, taken at 10 a.m. in animals fasting for 18 hours, were immediately centrifuged and serum was subdivided in aliquots and stored at -20°C until TLI determination, performed 72 hours after venepuncture. For the determination of TLI a polyclonal dog-specific double-antibody RIA kit, labeled with 125I, was used (DPC, Diagnostic Products Corporation, Los Angeles CA, USA, distributed in Italy by Medical Systems, Genoa). In all the samples the TLI concentration was determined in the bound fraction, obtained by centrifugation at +4°C and after supernatant aspiration. The samples were read in a computerized gamma counter with NaI (T1) detector (Canberra Packard, Meriden, CT, USA).

Results

The reference range obtained in present study is reported in Table 1. No variations related to sex were observed, except for a lightly higher standard deviation in males (Table 2).

As regards variations related to the different age groups, a sharp decrease in the serum concentration of TLI was observed between the group 3-3.5 months to the group 7-15 months (Fig. 2). The values then stabilized, and no significant variations in the subsequent age groups was observed.

The values (mean and standard deviation) obtained in the different groups under study are reported in Table 3.

The values of gastrin in different age groups, determined in the same animals in a previous study of the same authors, are reported for a comparison in Fig.3 (Cavallone and others, 1999). Given the importance of gastrin in regulating the synthesis of TLI, we compared the decrease of both parameters (TLI and gastrin) in percentage terms, calculated assuming the starting value as 100%. By this method a direct comparison of the trend of the two parameters in the time has been made possible, notwithstanding their different unit systems (Fig. 4).

### Table 1. TLI values (ng/ ml) in German shepherd dog sera

<table>
<thead>
<tr>
<th>Subjects number</th>
<th>Mean value</th>
<th>SD (standard deviation)</th>
<th>Reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>12.5</td>
<td>3.4</td>
<td>5.7 - 19.3</td>
</tr>
</tbody>
</table>

### Table 2. TLI values (ng/ ml) in both sexes

<table>
<thead>
<tr>
<th>Sex</th>
<th>Mean value</th>
<th>SD</th>
<th>Females Mean value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>males</td>
<td>12.3</td>
<td>4.6</td>
<td>females</td>
<td>12.6</td>
</tr>
</tbody>
</table>

### Table 3. TLI values (ng/ ml) in the different age groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean value</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1° (3-3.5 months)</td>
<td>15.9</td>
<td>3.7</td>
</tr>
<tr>
<td>2° (7-17 months)</td>
<td>9.3</td>
<td>1.6</td>
</tr>
<tr>
<td>3° (2-3 years)</td>
<td>12.2</td>
<td>3.3</td>
</tr>
<tr>
<td>4° (4-6 years)</td>
<td>11.3</td>
<td>3.9</td>
</tr>
<tr>
<td>5° (7-10 years)</td>
<td>11.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Groups: 1st = 3-3.5 months; 2nd =7-15 months; 3rd =2-3 years; 4th =4-6 years; 5th =7-10 years

Figure 2. TLI values (ng/ ml) in serum according to age.

Figure 3. Gastrin values (pg/ ml) in serum according to age.
The decrease observed between the first (3-3.5 months) and the second group (7-15 months) was identical, and was similar in the subsequent age groups.

Discussion and conclusions

The determination of TLI in serum samples is highly reliable and dog-specific (Archer and others, 1997; Boari and others, 1993; Williams and Batt, 1988). The population sample studied was statistically significant, and therefore appropriate to establish the TLI reference values in German shepherd dog. Such reference values are comparable to those reported in literature (5.2–35 ng/ml) also for other breeds, demonstrating that in the dog the breed does not influence the TLI serum concentration (Agugini and others, 1996; Archer and others, 1997; Boari and others, 1993; Braun, 1997). On the basis of present preliminary data we considered the TLI variations in the dog related to age, confirming trends already observed for PTH and gastrin (Cavallone and others, 1999). The growth of puppies is characterized by significant metabolic changes requiring by veterinary doctors to evaluate laboratory parameters carefully. The comparison between the proportional decrease of TLI and of gastrin in the time resulted was highly significant, confirming a suspected correlation of these two parameters in the dog. In fact also in this species the direct stimulatory activity of gastrin on the pancreatic secretion has been demonstrated (Le Drean and others, 1998; Nustede and others, 1993; Shulkes and Daldwin, 1997). The raised serum concentrations of both the parameters observed in the young subjects are probably related to the necessity to absorb proteins quickly, as they have an important function in puppies’ growth (Braun and others, 1997). With regard to gastrin, its main function is to stimulate the processes of digestion of proteins; the main function of TLI is the biochemical processing of proteins to allow absorption (Agugini and others, 1996; Faglia, 1997). The subsequent stabilization of these substances in the time, observed in adult animals, is probably related to the necessity to maintain a stable protein intake. As regards the sex of the dog, we did not observe any significant variation in healthy subjects. As the mechanisms regulating hormone secretions are very complex (e.g. their periodic secretion due to circadian cycles) the present study should be considered preliminary. Nevertheless we believe that it could be the basis for further investigations.

References


Johnson C.D., Chayvialle J.A. and others (1986) Neural pathways for the release of gastrin, cholecystokinin and pancreatic polypeptide after a meal in dogs. Digestive Diseases and Sciences; 31 (12); 1361-1369.


The European Journal of Comparative Gastroenterology publishes clinical and research papers on the medical and surgical aspects of small animal gastroenterology. Papers submitted for publication are reviewed by the Referees of the Editorial Board. Manuscripts and all communications should be sent to the editor:
Massimo Gualtieri, DVM, PhD
Istituto di Clinica Chirurgica e Radiologia Veterinaria - Università degli Studi di Milano - Via Celoria, 10 - 20133 Milano - Italy.
FAX: 39 02 70635010
E-mail: massimo.gualtieri@unimi.it
Manuscripts should be submitted in triplicate in English, typewritten (double spaced), with wide margins. All figures should be submitted in duplicate. The manuscript should also be provided on a computer disc (word x windows).

Format. Papers should be headed with the full title, which should be concise but informative, followed by the initials and surnames of the authors and full postal addresses. Each paper should have a summary (no more than 150 words) which embodies the main conclusions. The format can be flexible, but could include Introduction, Methods and Materials, Results, Discussion and Conclusions.

Tables, illustrations and photographs. Tables should be typewritten on separate sheets and numbered. Legends of illustrations and photographs should be typed on a separate sheet. Photographs can be colour or black and white. Each photograph should bear the author’s name and figure number in pencil on the back with the top marked with an arrow.

References. In the text the name of the author and the year should be in brackets, eg, (White, 1980). If the author’s name is an integral part of the sentence, the date only is placed in brackets, eg, as reported by White (1980). For more than two authors, (with and others, 1980) should be used. At the end of the paper the references should be listed in alphabetical order in the following form:

Journal Article
Author’s name(s) and initials, year of publication in brackets, full title of paper, full title of the journal, volume number and first and last page [eg, Abbott, D.P., Walsh, K & Diters, R.W. (1986) Calcyfing epithelial odontogenic tumours in three cats and a dog. Journal of Comparative Pathology 96, 131-136]

Chapter in a Book
Author’s name(s) and initials, date of publication in brackets, name of chapter or section, full title of book, edition, publishers and place of publication, pages referred to [eg, Peterson, M.E. (1995) Hyperthyroid disease. In: Textbook of Veterinary Internal Medicine, 4th edn. Eds. S.J. Ettinger and E.C. Feldman. W.B. Saunders, Philadelphia. pp 1466-1486]. Reprints will be sent to the first named author.

Articles in this Journal are indexed in the Index Veterinarius.

Advertisements bookings should be sent to:
Massimo Gualtieri,
Istituto di Clinica chirurgica veterinaria,
via Celoria 10
20133 Milano, Italy
FAX: 0039 02 70635010
E-mail: massimo.gualtieri@unimi.it

The European Society of Comparative Gastroenterology and the Editorial Board of the European Journal of Comparative Gastroenterology accept no responsibility for any omissions and/or errors in information printed in this journal. Any views and opinions expressed are those of the writer and not the European Society of Comparative Gastroenterology.