Some aspects of the effects of PL-10.1.AK-15 on the gastrointestinal tract

D Erceg, VN Simicevic, M Kolega, Cs Dohoczky

Summary — PL-10.1.AK-15 is an active fragment of a naturally occurring protein first isolated from human gastric juice. Among its other protective effects, PL-10.1.AK-15 has demonstrated a protective effect on the gastrointestinal tract. The aim of this study was to investigate the influence of PL-10.1.AK-15 on two functional parameters of gastrointestinal function: gastric acid secretion and gastrointestinal motility. Gastric acid secretion was assessed in male Wistar rats using a modified method of Shay, while gastrointestinal motility was assessed in male NMRI mice by charcoal propulsion. PL-10.1.AK-15 was given in three different doses (3, 10 and 100 μg/kg body weight) in accordance with the experimental protocol. The results of these experiments indicate that PL-10.1.AK-15 in the investigated doses had no influence on gastric acid secretion or gastrointestinal motility.

PL-10.1.AK-15 / body protection compound / gastric acid secretion / gastrointestinal motility / safety pharmacology

Introduction

The gastrointestinal tract is the site of toxic action for many drugs, first because it is the main route through which they are ingested (local toxic effects), and second because it is the target of their systemic actions (direct and indirect) (Improt and Broccardo, 1992). Drugs of totally unrelated chemical classes may share gastrointestinal side effects hampering adequate treatment of the original disease.

In the stomach, the most frequent drug-induced toxic effects consist of mucosal lesions of various degrees of severity: petechiae, superficial erosions or frank ulcers. Although the etiopathogenesis of gastric erosion is still unknown, the responsible factors are undoubtedly multiple: secretion and motility disorders, mucosal blood flow and mucosal barrier alterations (Isenberg et al., 1995). A change in one or more of these possible ethiological factors leads to a disequilibrium between conditions favoring aggression and protection, which is responsible for the appearance of erosions of the gastric mucosa and ulcers. This means that to assess the pharmacological safety of new molecules towards gastric ulceration, at least two of these parameters need to be studied concurrently (Improt and Broccardo, 1992). For this purpose, many experimental models are available. In general, they are designed to verify possible toxic side effects of drugs on the stomach and also to study the gastric cytoprotective effects of new molecules (Improt and Broccardo, 1992).

Recently, a novel protein was isolated from human gastric juice and called body protection compound (BPC) (Sikiric et al. 1991a, b). The protein was partially amino sequenced from its N-terminal end resulting in the synthesis of a pentadecapeptide with a molecular mass of 1419.5 Da. This pentadecapeptide is presently coded PL-10.1.AK-15 but also carries prior codes of BPC 157 (Paré and Kluczynski, 1992; Sikiric et al. 1994) and BPC 15 (Veljaca et al., 1995). Both the protein and synthesized peptide have shown protective effects in various animal models of disease (Sikiric et al. 1991a, b, 1993, 1994; Mózsik et al., 1991; Veljaca et al., 1995; Sandor et al., 1996). Among its protective effects, PL-10.1.AK-15 has demonstrated a protective effect in the gastrointestinal tract, particularly in the development of acute gastric lesions (Paré and Kluczynski, 1992; Sikiric et al. 1994; Sandor et al. 1996). The aim of this study was, therefore, to investigate the effects of PL-10.1.AK-15 on two functional gastrointestinal parameters: gastric acid secretion and gastrointestinal motility and to see whether the gastroprotective action of PL-10.1.AK-15 was due to changes in these two parameters.

Materials and methods

Gastric acid secretion was assessed using a modified Shay method. Male Wistar rats (home bred), weighing 220–330 g, were housed under standard conditions (temperature 22 ±2°C; humidity 55 ±5%; 12 h light-dark cycle) and divided, randomly, into four experimental groups (eight rats per group). Following 18 h of fasting, a midline laparatomy was performed under light ether anesthesia and the pylorus was ligated according to a modified version of the Shay method (Shay et al., 1954). Three experimental groups received PL-10.1.AK-15 (supplied by Diagen, Slovenia) in doses of 3, 10 and 100 μg/kg body weight per os, respectively, immediately following ligature. The fourth group served as a control and received an equal volume of saline in the same regimen. Five h following agent administration, the animals...
were killed and gastric juice was collected. Following centrifugation, the volume of the supernatant, pH and the total acidity were measured.

Gastrointestinal motility was measured in the following manner. Four randomly assigned experimental groups of male NMRI mice (home bred), weighing 21-29 g (10 mice per group), were housed under standard conditions (temperature 22 ± 2°C; humidity 55 ± 5%; 12 h light-dark cycle) and fasted for a period of 18 h. Three groups received PL-10.1.AK-15 (supplied by Diagen, Slovenia) in doses of 3, 10 and 100 µg/kg body weight per os, respectively, 30 min prior to the experimental procedure. The fourth group served as a control and received an equal volume of saline in the same regimen. Gastrointestinal motility was measured using charcoal propulsion (5% solution of medical charcoal in a 10% solution of Arabic gum) (Hirotsu et al., 1988). The length of the gut in mm (GL), as well as the length of the charcoal passage in mm (CP) from the pylorus to the distance traveled by the charcoal meal were measured. Intestinal transit (CP/GL x 100) was compared.

All results are expressed as means ± SEM. The difference between means was compared using analysis of variance (ANOVA) on the SAS program. Differences were considered significant at a level of P < 0.05.

Results

All results are shown in tables I and II. Briefly, PL-10.1.AK-15 in the investigated doses had no effect on either of the investigated parameters. Namely, no statistical difference could be noted in either of the experimental groups when PL-10.1.AK-15 treated animals were compared to vehicle-treated control groups.

Discussion and conclusion

PL-10.1.AK-15 has been reported to show antiulcerative effects in various models of peptic ulcer disease (Sikric et al., 1993, 1994; Sandor et al., 1996). In the present study, the effects of PL-10.1.AK-15 on gastric acid secretion and gastrointestinal motility were investigated.

Table I. The effect of PL-10.1.AK-15 on gastric secretion, pH and total acidity.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Volume of gastric secretion (mL ± SEM)</th>
<th>pH (± SEM)</th>
<th>Total acidity (mmol/L ± SEM)/5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>16.06 ± 1.82</td>
<td>1.96 ± 0.33</td>
<td>0.17 ± 0.02</td>
</tr>
<tr>
<td>PL 10.1.AK15</td>
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<tr>
<td>10 µg/kg</td>
<td>15.50 ± 2.28</td>
<td>1.65 ± 0.07</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>30 µg/kg</td>
<td>15.13 ± 1.92</td>
<td>1.57 ± 0.04</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>100 µg/kg</td>
<td>18.63 ± 2.34</td>
<td>1.68 ± 0.05</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

Both the Shay pylorus ligation model and the charcoal propulsion model are standard tests used in the safety evaluation of potential therapeutic agents (Improta and Broccardo, 1992).

The pylorus ligation procedure is a means of testing rat gastric secretion and is also widely used for studying antulcer agents. Ligation induces a continuous secretion with high acid concentration which appears to be maximal since secretion is never further increased by injections of pentagastrin (Brodie, 1966). This method also allows measurements of the composition of gastric juice.

In the genesis of peptic ulceration, the relationship between the H+ and the rate of flow of gastric juice may have a greater potential. Drugs increasing gastric flow more and acid output less do not produce gross damage to the stomach, whereas drugs decreasing gastric volume and increasing H+ are ulcerogenic (Improta and Broccardo, 1992). The relationship between H+ and flow of gastric juice is similar during stimulation of gastric acid secretion by gastrin and histamine (Alumets et al., 1982). Moreover, this relationship is not altered during inhibition of gastric acid secretion by histamine H2 antagonists. The results obtained in this study clearly indicate that PL 10.1.AK-15 in the applied doses did not alter acid secretion or H+ content (table I).

It is generally accepted that in some types of ulcers, particularly those induced by stress, the major causal role is played not by the amount of acid, but by increased gastric motility (Szabo, 1989). Motility changes seem to contribute to the pathogenesis of both gastric and duodenal ulceration. For this reason, assays examining the safety of a drug towards the gastrointestinal tract should always include tests of motility, because changes in this function might provoke future gastrointestinal disease.

Muscular activity, contractions of the intestinal wall and transit of contents represent the main motor functions in the GI tract. Transit, therefore, is the result of processes of mixing, propulsion and retropropulsion that are present to different degrees.
in the various parts of the gastrointestinal tract. Because no single technique can evaluate all the various aspects of transit at each level of the alimentary tract, various techniques have to be employed. To measure gastrointestinal motility accurately the ideal marker should be chemically, physiologically and pharmacologically inactive. Thus the marker used in our present study was medical charcoal. The results of this study point to the fact that PL-10.1.AK-15 in the investigated doses did not show any statistically significant difference when compared to the control animal (table II).

Pharmacological modulation of cellular and vascular factors in the stomach provides a potential approach in the medical treatment or prevention of erosions and ulcers in this organ. Until two decades ago, such a comprehensive approach to ulcer disease was unthinkable since ulcer therapy had historically been associated with neutralization or inhibition of gastric acid secretion. The discovery of drugs which are virtually ineffective towards gastric acid was parallel with the concept of gastric "cytoprotection".

To conclude, the results of our study clearly indicate that PL-10.1.AK-15, when administered in three different doses per os, does not possess any influence on gastric acid secretion or gastrointestinal motility, two parameters of gastrointestinal function which are used in the safety assessment of potential therapeutic agents. Its protective effects, exerted in the stomach and described by other authors, is not, therefore, mediated through these two possible mechanisms.

Acknowledgments

The authors wish to thank Mrs Anica Pesut and Mr Zeljko Javorosbak for their technical assistance in the experimental procedures.

Table II. The effect of PL-10.1.AK-15 gastrointestinal motility (charcoal passage).

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Mean distance traveled by charcoal as % of the total gut length (± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL 10.1.AK15</td>
<td>49.20 ± 4.17</td>
</tr>
<tr>
<td>10 µg/kg</td>
<td>51.61 ± 3.15</td>
</tr>
<tr>
<td>30 µg/kg</td>
<td>62.66 ± 4.12</td>
</tr>
<tr>
<td>100 µg/kg</td>
<td>56.74 ± 3.09</td>
</tr>
</tbody>
</table>

*P < 0.05 ANOVA; SEM, standard error of means.

References