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New model of cytoprotection/adaptive cytoprotection in rats: endogenous small irritants, antiulcer agents and indomethacin

Predrag Sikirić *, Sven Seiwerth, Slobodan Dešković, Zeljko Grabarević, Anton Marović, Rudolf Ručman, Marijan Petek, Paško Konjevoda, Stipislav Jadrijević, Tomislav Šoša, Darko Perović, Gorana Aralica, Branko Turković

Center for Digestive Diseases, Medical and Veterinary Faculty, University of Zagreb, Zagreb, Croatia

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Abstract

Adaptive cytoprotection in the stomach was originally defined by applying the exogenous irritants only. The contribution of endogenous irritants as inductors of initial lesions was not specially evaluated. No attempt was made to either focus antiulcer agent activity on adaptive cytoprotection, or split their 'cytoprotection' into complex adaptive cytoprotective activity and simple cytoprotective effects. Agents had so far not been applied simultaneously with the second challenge with ethanol (or irritant), when differences between cytoprotection and adaptive cytoprotection appear. Gastrojejunal anastomosis for 24 h in rats was introduced as new model for analyzing cytoprotection/adaptive cytoprotection. The contribution of the up-normal level of endogenous irritants and the endogenous small irritant-induced minor lesions during the adaptive cytoprotection were studied. The effect of late challenge with 96% ethanol in the presence of an up-normal level of endogenous irritants and endogenous small irritant-induced minor lesions was compared with results of classic studies of ethanol-induced gastric lesions in normal rats (1 ml/rat i.g.). Antiulcer agents or a prostaglandins-synthesis inhibitor, indomethacin, given once only in classic studies, were given at several points during injury induction: (i) surgery, (ii) mild ethanol, (iii) strong ethanol, (iv) strong ethanol applied after a suitable period following either mild ethanol or surgery). Their effects were compared in rats treated as follows: exogenous irritant studies (96% or 20% ethanol), exogenous/exogenous irritant studies (20% ethanol 1 h before 96% ethanol), endogenous irritant studies (gastrojejunal anastomosis for 24 h), and endogenous/exogenous irritant studies (gastrojejunal anastomosis for 24 h before 96% ethanol). Characteristic of the various irritants differed: the (preceding) small irritants (exogenous (i.e., mild ethanol in healthy intact rats) (exogenous irritant studies) vs. endogenous (e.g., (increased) gastric acid secretion, duodenal reflux in gastric content in rats with termino-lateral gastrojejunal anastomosis) (endogenous irritant studies)). These factors caused modifications of agents' activities not, as initially thought, giving simple 'cytoprotection', but being only cytoprotective, or adaptive cytoprotective, or both cytoprotective and adaptive cytoprotective. Atropine (10 mg/kg i.p.) and ranitidine (10 mg) had only cytoprotective activity (exogenous irritant-studies), whereas pentadecapeptide BPC157 (10 μg or 10 ng), and omeprazole (10 mg) had mainly adaptive cytoprotective activity (endogenous/exogenous irritant studies) or both cytoprotective and adaptive cytoprotective activities (exogenous/exogenous irritant studies). Augmentation of the lesions by indomethacin (5 mg/kg s.c.), showed that only events preceding the late challenge with ethanol may be prostaglandin-dependent in both models. The second, adaptive cytoprotective part, seen after late ethanol challenge, may be either prostaglandin-dependent (exogenous/exogenous irritant studies) or non-dependent (endogenous/exogenous irritant studies). Both spontaneous lesion reduction, as an essential mechanism of adaptive cytoprotection, and the further lesion reduction by agents, such as pentadecapeptide BPC 157 and omeprazole, suggests that these agents function as an essential link between the various reactions in cytoprotection/adaptive cytoprotection.

Keywords: Irritant, endogenous, small; Cytoprotection; Cytoprotection, adaptive; Surgery; Anastomosis; Pentadecapeptide BPC 157; Omeprazole; Antiulcer agents; (Rat)

* Corresponding author. Department of Pharmacology, Medical Faculty, University of Zagreb, Salata 11, P.O. Box 916, 10000 HR Zagreb, Croatia. Tel.: +385-1-4566-833, +385-1-4566-834, +385-1-4566-836; Fax: +385-1-424-001, +385-1-4683-829; E-mail: psikiric@mef.hr

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1. Introduction

Based on Robert's intriguing concept of cytoprotection (gastric acid independent protection after one irritant challenge (exogenous irritant studies)) and adaptive cytoprotection, i.e., small irritants and small lesions protect against strong irritants and strong lesions in the stomach (Robert, 1979; Robert et al., 1983; Robert, 1985; Robert et al., 1991), many agents were claimed to be cytoprotective (Moron et al., 1984; Szabo et al., 1985; Clissold and Campoli-Richards, 1986; Szabo, 1986, 1989; Sikiric et al., 1993b, 1994, 1995; Kato et al., 1996). Based on results of recent research in cytoprotection (Sikiric et al., 1997c) a complete lack of gastric acid secretion in rats was created by total gastrectomy and challenged with an ulcerogenic dose of cystamine. As a the result, long-claimed cytoprotective effect of many agents may eventually prove to be completely gastric acid-independent, and the existence of cytoprotection (Robert's independence from gastric acid) may be properly validated (Sikiric et al., 1997c). However, in this light, several aspects of Robert's adaptive cytoprotection still need elucidation.

It is claimed by Robert et al. (1983) and Robert (1985) that the influence of natural endogenous small irritants, e.g., (increased) gastric acid secretion, duodenal reflux of gastric content, disturbed motility—as assumed in ulcer disease (Robert, 1979; Robert et al., 1983; Moron et al., 1984; Robert, 1985; Szabo et al., 1985; Clissold and Campoli-Richards, 1986; Szabo, 1986, 1989; Robert et al., 1991; Kato et al., 1996), is responsible for minor lesions and initiation of a defensive response against major irritant strong lesions. This assumption should be explored directly, which could be hardly done in classic studies. Namely, classic studies of adaptive cytoprotection in normal rats (application of exogenous irritant, e.g., mild alcohol (mild irritant) prior strong irritant, e.g., strong alcohol (exogenous/exogenous irritant studies)) (e.g., see Robert et al., 1983; Robert, 1985) could not anticipate the sustained induction of endogenous (mild) irritants, and the need for up-normal levels for small-lesion induction. If adaptive cytoprotection is initiated by an initial instillation of exogenous mild irritant, it is a short-term phenomenon, with brief presence in the stomach as limitation (Robert et al., 1983). Acute administration of an exogenous mild irritant as a 'small irritant' as opposed to a sustained increased level of endogenous irritants precludes generalization of the results to adaptive cytoprotection. Theoretically, unlike the challenge with mild alcohol (Robert et al., 1983), an (increased) gastric acid secretion, duodenal reflux in gastric content, or disturbed motility as endogenous mild irritants could potentiate rather than attenuate the effect of subsequent strong alcohol application. Consequently, suitable surgery is needed for their sustained induction (endogenous irritant studies). This could permit the investigation of possible effects of the application of strong irritant under conditions better mimicking disturbed conditions, e.g., ulcer disease, under which activity of the corresponding endogenous irritants could be important (endogenous/exogenous irritant studies). So far, such a demonstration of adaptive cytoprotection is still lacking.

Also, no attempt has been made to focus the activity of the antiulcer agents onto adaptive cytoprotection. Agents have so far been combined with the first ethanol challenge (Robert et al., 1983; Robert, 1985; Kato et al., 1996), and were not applied at the time of the second application of ethanol (or irritant), when the difference between cytoprotection (Robert, 1979) and *adaptive* cytoprotection (Robert et al., 1983) may begin, i.e., ethanol (Robert, 1979) vs. ethanol + *ethanol* (Robert et al., 1983). The agents combined with the second ethanol challenge would be more likely influence only the adaptive cytoprotection defense response, with no effect on the prior cytoprotective response, i.e., had they been linked with first ethanol challenge (Robert et al., 1983; Robert, 1985).

Likewise, no attempt was done to split the 'cytoprotection' agents into more complex adaptive cytoprotection and/or simple cytoprotective effects. Probably combining the results of experiments with endogenous irritants, and endogenous/exogenous irritants with results of classic exogenous irritant experiments with results from an exogenous/exogenous irritant model, could reveal that an agent with cytoprotective properties does not necessarily have an inherent adaptive cytoprotective activity.

In this, we introduced gastrojejunal anastomosis for 24 h in rats as a new model for analysis of cytoprotection/adaptive cytoprotection. Furthermore, in present study, instead of using only one medication at only one arbitrary point as in classic studies (Robert et al., 1983; Robert, 1985), the antiulcer agents or a prostaglandin-synthesis inhibitor, indomethacin, were given at several points in injury induction, and their effect in exogenous irritant-, exogenous/exogenous irritant-, endogenous irritant-, and endogenous/exogenous irritant-experiments were compared. All these antiulcer agents (stomach pentadecapeptide BPC 157, ranitidine, atropine, omeprazole) were suggested to have also a cytoprotective activity (Moron et al., 1984; Clissold and Campoli-Richards, 1986; Sikiric et al., 1993b, 1994, 1995, 1996b, 1997c). They have further more recently been shown to have direct cytoprotective activity also, e.g., they protected even gastrectomized rats against cystamine-duodenal lesions (Sikiric et al., 1997c).

2. Materials and methods

2.1. BPC 157: Preparation of the peptide

Pentadecapeptide Gly-Glu-Pro-Pro-Pro-Gly-Lys-Pro-Ala-Asp-Asp-Ala-Gly-Leu-Val, M.W. 1419, named BPC 157 which is a part of the sequence of human gastric juice protein, coded BPC, freely soluble in water at pH 7.0 and in saline, was prepared as described before. The peptide with 99% (high pressure liquid chromatography, HPLC)

purity (1-des-Gly peptide as impurity, biologically inactive) was used (Sikiric et al., 1993a,b, 1994, 1995, 1996b, 1997a,b,c,d,e; Seiwerth et al., 1997).

2.2. Animals

Male Albino Wistar rats, 200 g b.wt., randomly assigned to groups, were used for the experiments, approved by local committee. The ethanol procedure was done after overnight fasting, with water provided until 2 h before instillation. Besides, to avoid misinterpretations due to a possible dilution of the strong ethanol by the gastric contents, animals allowed water ad libitum, were used as additional control.

2.3. Experimental procedure

2.3.1. Exogenous irritant

2.3.1.1. Ethanol models. Ethanol 96% or 20% was given intragastrically (i.g.) in a dose of 1.0 ml/rat as previously described (Sikiric et al., 1994, 1996b). The animals were killed 60 min after ethanol (see Fig. 1).

2.3.2. Exogenous / exogenous irritant

Alternatively, ethanol was given as part of the protocol including the administration of the 20% ethanol (i.g. in a dose of 1.0 ml/rat) 60 min before 96% ethanol (i.g. in a dose of 1.0 ml/rat), the animals were killed 60 min after the application of the strong ethanol (see Fig. 1).

2.3.3. Endogenous irritant

To induce endogenous irritants' up-normal level, e.g., (increased) gastric acid secretion, duodenal reflux in gastric content, etc., the surgical procedure was done under ether anesthesia after overnight fasting, with water available until surgery. After the duodenum was cut and closed 15 cm from the end of pylorus, the stomach at the glandular part of the greater curvature, and the aboral part of the jejunum were joined by a termino-lateral anastomosis. The animals were killed 24 h after surgery (they were without food after surgery, with water up to the last 2 h before killing) (see Fig. 1).

2.3.4. Endogenous / exogenous irritant

Rats with gastrojejunal termino-lateral anastomosis, and blind closure of the duodenum, and with an up-normal level of endogenous irritants, 24 h after surgery, were challenged with 96% ethanol (i.g. in a dose of 1.0 ml/rat). They were killed 1 h after ethanol instillation. The animals were left without food after surgery, but had water up to 2 h before ethanol application (see Fig. 1).

2.3.5. Drugs and drugs application

The medication (/kg b.wt.): the beneficial agents: pentadecapeptide BPC 157 10 μg or 10 ng, atropine (atropine sulfate, Sigma, USA) 10 mg, ranitidine (Ranital, Pliva, Croatia) 10 mg, omeprazole (Ultop, Krka, Slovenia) 10 mg, intraperitoneally (i.p.); a prostaglandin synthesis inhibitor: indomethacin 5.0 mg, subcutaneously (s.c.) or an equal volume of saline (5 ml) was given either 1 h before

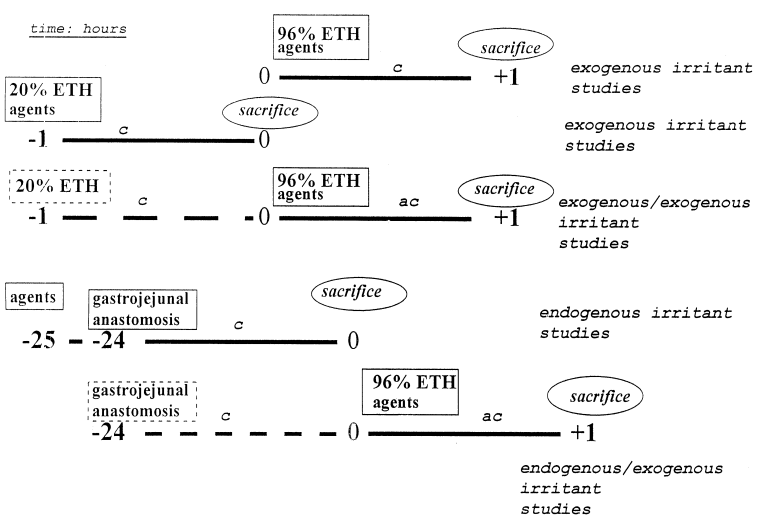

Fig. 1. Scheme of the regimens. Time (0) calculated from the moment of strong irritant (96% ethanol) application, or killing. Time (−) indicated the events prior to late challenge with ethanol or the intervals before killing (in the studies without strong irritant application). Time (+) calculated from the moment of late challenge with strong irritant (96% ethanol) application. ETH—ethanol; agents—salutary agents: pentadecapeptide BPC 157 10 μg or 10 ng; atropine 10 mg, ranitidine 10 mg, omeprazole 10 mg (i.p.); prostaglandins synthesis inhibitor indomethacin 5 mg s.c. Controls received an equal volume of saline (5 ml/kg b.wt., i.p.). 10–15 rats per experimental group; c—period after one ethanol administration, or endogenous irritants induction by anastomosis, referred as cytoprotection; ac—period referred as adaptive cytoprotection, after late ethanol administration, in the rats that had been challenged before by exogenous irritant instillation, or by induction of endogenous irritants by anastomosis (meanwhile period referred as cytoprotection-period (c)).

surgery (endogenous irritant studies), or simultaneously with ethanol application (exogenous irritant studies, exogenous/exogenous irritant studies, endogenous/exogenous irritant studies). All the antiulcer agents were dissolved in saline, except to omeprazole (prepared as before; Sikiric et al., 1997c), and indomethacin (Indocid, Lek, Slovenia), which was given as a suspension in 1% sodium carboxymethyl cellulose solution (Sikiric et al., 1996b).

2.3.6. Assessment of mucosal injury

Immediately after killing of the rats, the stomach was removed and the lesions (mm^2, means ± SEM) were assessed by naive observers as described before (Sikiric et al., 1993b, 1994, 1996b). Representative sections of the stomach were processed for further histological analysis. The lesions induced in rats with gastrojejunal terminolateral anastomosis and the ethanol-induced lesions were assessed by microscopy as described before (Sikiric et al., 1993b, 1994, 1996b) with a morphometric analysis system (using a PC-based program SFORM, VAMS, Zagreb, Croatia). Representative tissue sections were processed for further histological analysis as described before.

2.3.7. Statistical analysis

A Kolmogorov–Smirnov test was applied for estimation of the normality of the data distribution. Further statistical analyses were done by means of analysis of variance (ANOVA) and/or Kruskal–Wallis test, Student–Newman–Keuls, Dunn's and Dunnett's tests. Differences of 0.05 or less were considered statistically significant.

3. Results

It is important for the definition of cytoprotection/adaptive cytoprotection (Robert, 1979; Robert et al., 1983; Robert, 1985; Robert et al., 1991) to note that adaptive cytoprotection may be noticeable only after (but not before) the repeated challenge with ethanol. Therefore, if adaptive cytoprotection is the aim, application of agents with the first instillation of irritant, i.e., ethanol (Robert et al., 1983; Robert, 1985; Kato et al., 1996) may be premature and inappropriate, contrasting with theoretical definition (Robert, 1979; Robert et al., 1983; Robert, 1985; Robert et al., 1991), making any conclusions misleading. Thus, to demonstrate the possible cytoprotective–adaptive cytoprotective (dis)connection in the activity of the antiulcer agents, instead of using only one medication at one arbitrary point, we gave the agents (stomach pentadecapeptide BPC 157, ranitidine, atropine, omeprazole) or a prostaglandins-synthesis inhibitor, indomethacin, at various moments of injury induction (1 h before surgery or simultaneously with each ethanol application, i.e., 96% or 20%). To clarify this issue a number of protocols (see Sections 2.3.1, 2.3.2, 2.3.3 and 2.3.4) were used, in addition to administration of the prostaglandin synthesis inhibitor, indomethacin, and a variety of the beneficial agents.

3.1. Exogenous irritant experiments

3.1.1. Application of 96% ethanol only

Consistent with accepted evidence, the intragastric application of strong ethanol consistently produced severe lesions in all the control rats. All the agents tested were shown to potently inhibit the otherwise severe ethanol-induced lesions. Indomethacin administration: there was consistent aggravation of the ethanol-induced lesions in all rats after subcutaneous co-administration of indomethacin with the ethanol intragastric instillation (Figs. 1 and 2).

3.1.2. Application of 20% ethanol only

The mild ethanol produced no more than minute lesions in the control rats. Simultaneous administration of the beneficial agents, pentadecapeptide BPC 157, ranitidine, atropine, omeprazole consistently reduced these lesions. Indomethacin administration: as in the rats given strong ethanol-damaged, and killed 1 h after mild ethanol administration, a subcutaneous co-administration of indomethacin with mild ethanol intragastric instillation also resulted in a consistent aggravation (Figs. 1 and 3).

Thus, in these experiments, along with the suggested cytoprotective activity of the agents tested alone (Moron et al., 1984; Clissold and Campoli-Richards, 1986; Sikiric et al., 1997c), there was cytoprotective activity (against either strong ethanol or mild ethanol) by all tested agents. In view of aggravation of the ethanol-induced lesions by indomethacin, this activity seems to be at least partly prostaglandin-dependent, which is consistent with general knowledge about cytoprotection (Robert, 1979; Robert et al., 1983; Moron et al., 1984; Robert, 1985; Szabo et al., 1985; Clissold and Campoli-Richards, 1986; Szabo, 1986,

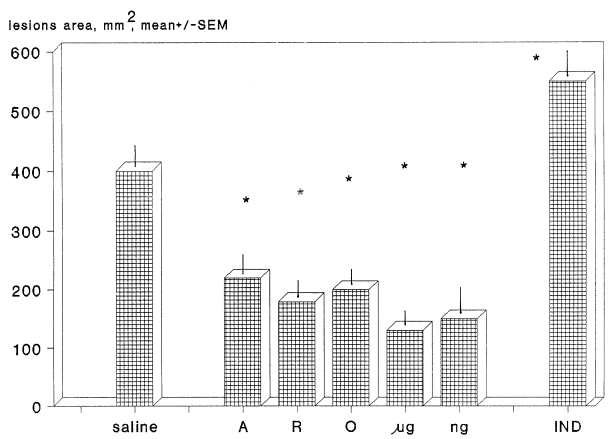

Fig. 2. Exogenous irritant studies. Stomach lesions induced by an intragastric application of 1 ml/rat of the 96% ethanol, and assessed 1 h thereafter. Treatment (/kg b.wt.) simultaneously with ethanol (salutary agents: pentadecapeptide BPC 157 10 µg (µg) or 10 ng (ng), atropine 10 mg (A), ranitidine 10 mg (R), omeprazole 10 mg (O) i.p.; prostaglandin synthesis inhibitor indomethacin (IND) 5 mg s.c.). Controls received an equal volume of saline (5 ml/kg b.wt., i.p.). 10–15 rats per experimental group. * $P < 0.05$, at least vs. control.

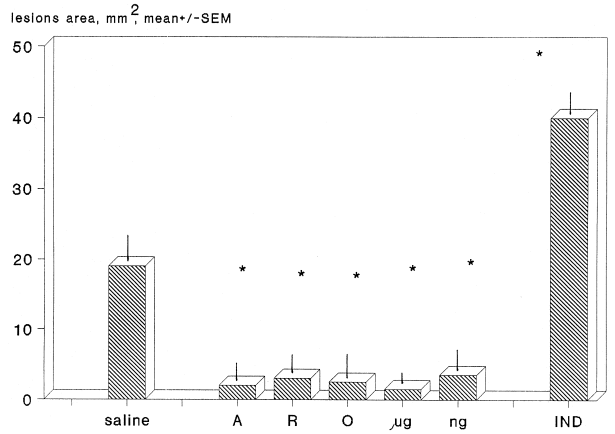

Fig. 3. Exogenous irritant studies. Stomach lesions induced by intragastric application of 1 ml/rat of the 20% ethanol, and assessed 1 h thereafter. Treatment (/kg b.wt.) simultaneously with ethanol (salutary agents: pentadecapeptide BPC 157 10 μg (μg) or 10 ng (ng), atropine 10 mg (A), ranitidine 10 mg (R), omeprazole 10 mg (O) i.p.; prostaglandin synthesis inhibitor indomethacin (IND) 5 mg s.c.). Controls received an equal volume of saline (5 ml/kg b.wt. i.p.). 10–15 rats per experimental group. $* P < 0.05$, at least vs. control.

1989; Robert et al., 1991; Sikiric et al., 1993b, 1994, 1995, 1996b; Kato et al., 1996).

3.2. Exogenous / exogenous irritant experiments

3.2.1. Mild ethanol application followed by strong ethanol administration

Given 1 h after a mild irritant, according to the Robert et al. (1983) adaptive cytoprotection protocol, when the small irritant-induced lesions were already established, strong ethanol produced smaller lesions than it had in exogenous irritant studies, i.e., if the same challenge with strong ethanol had been applied without pretreatment with mild ethanol. Indomethacin administration: subcutaneous co-administration of indomethacin with late ethanol intragastrical instillation resulted in consistent aggravation of the ethanol lesions in all rats (Figs. 1 and 4).

Using lesion size as the only criterion of activity, the adaptive cytoprotection-strong ethanol-induced lesions (Robert et al., 1983), should be further reduced in the exogenous/exogenous irritant protocol by the agents known to antagonize the greater lesions seen in the exogenous irritant protocol. This would apply unless different quality of the final lesions were at different type. Accordingly, in rats pretreated with mild ethanol, only pentadecapeptide BPC 157 and omeprazole applied alternatively with the strong ethanol, further reduced these lesions, to below the mild + strong ethanol control values. Atropine and ranitidine did not reduce lesion formation below the mild + strong ethanol control values.

These data clearly showed that, i.e., atropine, ranitidine, the beneficial effects of the antiulcer agents could sometimes be changed over the cytoprotection–adaptive cytoprotection course, most likely, at the breakpoint of cytoprotection vs. adaptive cytoprotection (effectiveness against strong ethanol lesions and effectiveness against mild ethanol lesions (exogenous irritant studies) vs. non-activity against strong ethanol after mild ethanol (exogenous/exogenous irritant studies)). Since indomethacin aggravated the lesions induced by strong ethanol given after mild ethanol, as it did in exogenous irritant studies, these alterations in the beneficial activity of atropine and ranitidine in exogenous/exogenous irritant studies could suggest that their action does not involve prostaglandins. On the other hand, it is also possible that pentadecapeptide BPC 157 and omeprazole participate in the entire process of both cytoprotections, Robert's cytoprotection (as an early process) and adaptive cytoprotection (as a subsequent, later process). Besides, it is known that systems other than prostaglandins are involved in cytoprotection and adaptive cytoprotection (Szabo et al., 1985; Szabo, 1986, 1989; Kato et al., 1996).

3.3. Endogenous irritant experiments

3.3.1. The effects on the lesions development after 24 h of gastrojejunal anastomosis

Cutting and closure of the duodenum at 15 cm from the end of the pylorus, and direct connection of the stomach, at the glandular part of the greater curvature, with the aboral part of the jejunum, produced by a termino-lateral anastomosis, induced conditions in gastrointestinal tract that could mimic likely disturbances in ulcer disease: a distended stomach and duodenum full of their content, i.e., an increased accumulation of gastric acid, and duodenal

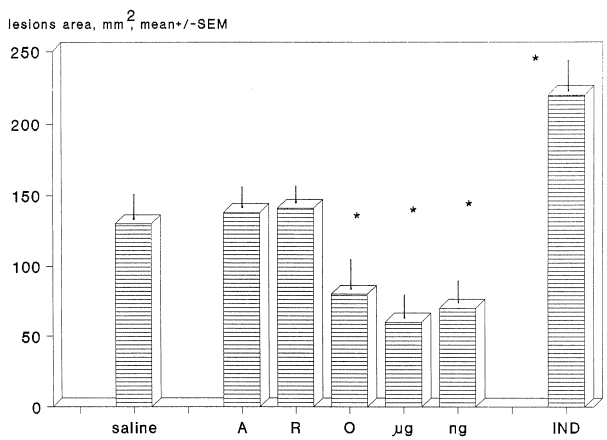

Fig. 4. Exogenous/exogenous irritant studies. Stomach lesions induced by an intragastric application of 1 ml/rat of 96% ethanol, and assessed 1 h thereafter. The rats received 20% ethanol 1 ml/rat i.g. 1 h before 96% ethanol 1 ml/rat i.g. Treatment (/kg b.wt.) simultaneously with 96% ethanol (salutary agents: pentadecapeptide BPC 157 10 μg (μg) or 10 ng (ng), atropine 10 mg (A), ranitidine 10 mg (R), omeprazole 10 mg (O) i.p.; prostaglandin synthesis inhibitor indomethacin (IND) 5 mg s.c.). Controls received an equal volume of saline (5 ml/kg b.wt., i.p.). 10–15 rats per experimental group. $* P < 0.05$, at least vs. control.

reflux, due to stasis and duodenal obstruction. These conditions are in some respect similar to the pylorus ligation model, but unlike pylorus-ligated rats (Sikiric et al., 1995), these animals have an uninterrupted communication between stomach and duodenum, and the blood supply not compromised by the ligature. Under these conditions, 24 h after anastomosis, the lesions appeared predominantly in the stomach (Figs. 1 and 5).

The consistent lesion development clearly implies some intrinsic ulcerogenic potential of the up-normal level of endogenous irritants (volume content cc 10 ml, pH 5.5). The lesions, as compared with those seen following application of strong ethanol alone, could be considered as minor lesions. These lesions could be thus suitable for further investigation of the effect of later application of strong irritant (see below).

Intriguingly, application of the agents, atropine, ranitidine and omeprazole augmented these lesions. Pentadecapeptide BPC 157 did not influence the appearance of these lesions. Indomethacin administration: subcutaneous administration of indomethacin before surgery consistently aggravated the gastrojejunal anastomosis lesions in all rats.

The lesions appeared mostly in the glandular part of the stomach, at a site distant from the anastomosis. Some lesions would also appear close to the site of anastomosis and in the upper part of the jejunum, whereas the duodenum was almost completely spared.

These findings clearly evidenced that the minor lesions appeared after the level of the endogenous irritants induced by gastrojejunal anastomosis. The demonstration that these lesions were aggravated by indomethacin, i.e., prostaglandin-synthesis inhibition, as they were by the antiulcer agents (except to pentadecapeptide BPC 157) may merit particular attention, i.e., mechanism(s) other than prostaglandins. Nevertheless, the aggravation by indomethacin and the subsequent relative attenuation of otherwise more severe lesions induced by strong ethanol administration (see below), may be taken as indicators of adaptive cytoprotection. The conditions for activation of endogenous adaptive (cyto)protection could be triggered by a disturbed gastrointestinal balance, and these processes could be highly advanced at the time of strong irritant, i.e., strong ethanol, application. The antiulcerogenic agents, such as atropine, ranitidine, omeprazole—due to their inhibition of gastric acid secretion (see Moron et al., 1984; Clissold and Campoli-Richards, 1986) thus could partly interfere with this essential, initial endogenous protective response, and would, paradoxically, even aggravate the lesions. The pentadecapeptide, unlike others agents, would not interfere as it has no effect on gastric acid secretion (Sikiric et al., 1993b; Erceg et al., 1997).

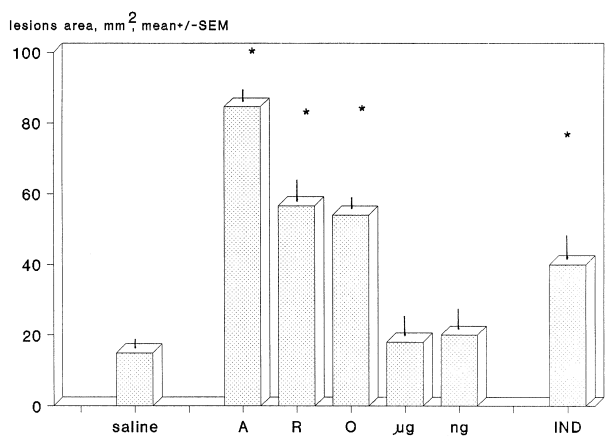

Fig. 5. Endogenous irritant studies. Stomach lesions induced by surgery (gastrojejunal termino-lateral anastomosis, with blind closure of the duodenum), and assessed 24 h thereafter. Treatment (/kg b.wt.) 1 h before surgery (salutary agents: pentadecapeptide BPC 157 10 µg (µg) or 10 ng (ng), atropine 10 mg (A), ranitidine 10 mg (R), omeprazole 10 mg (O) i.p.; prostaglandin synthesis inhibitor indomethacin (IND) 5 mg s.c.). Controls received an equal volume of saline (5 ml/kg b.wt., i.p.). 10–15 rats per experimental group. $* P < 0.05$, at least vs. control.

The above discussion together with the findings from exogenous irritant-experiments (i.e., lesion reduction with all antiulcer agents), suggests that the beneficial processes occurring during accumulation of the endogenous small irritants in the stomach of the rats with anastomosis are different and not further potentiated by antiulcerogenic agent application.

3.4. Endogenous / exogenous experiments

3.4.1. The effects seen on challenge with strong ethanol and gastroenteroanastomosis

Whereas strong ethanol applied alone in exogenous irritant in non-operated rats without a preceding challenge with mild ethanol produced severe lesions, the anastomosis + strong ethanol-lesions in operated control rats were markedly less pronounced. Interestingly, the antiulcer agents further attenuated in the rats with gastrojejunal anastomosis the endogenous/exogenous irritant-induced lesions, for example, pentadecapeptide BPC 157, in either dose, or omeprazole was given. This had already been seen in exogenous/exogenous irritant experiments. Atropine did not reduce the lesions below the values in control rats with gastrojejunal anastomosis, whereas ranitidine potentiated the endogenous/exogenous irritant-induced lesions. Indomethacin administration: subcutaneous co-administration of indomethacin with the late intragastric instillation of strong ethanol did not influence these lesion levels (Figs. 1 and 6).

Thus, with the mild lesions seen in the stomach following gastrojejunal anastomosis, the finding of marked attenuation of the otherwise severe lesions induced by strong ethanol in endogenous/exogenous irritant experiments strongly favors the possibility of an adaptive cytoprotection phenomenon. However, if responsiveness or non-responsiveness to indomethacin is taken as a basis for defining cytoprotection/adaptive cytoprotection lesions (Robert et al., 1983; Robert, 1985), the lesions now found in endogenous irritant-and endogenous/exogenous irritant-

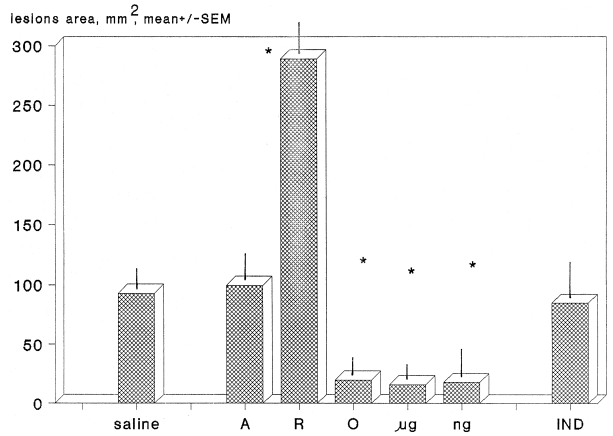

Fig. 6. Endogenous/exogenous irritant studies. Stomach lesions induced by intragastrical application of the 1 ml/rat of 96% ethanol, and assessed 1 h thereafter. The rats were subjected to surgery (gastrojejunal terminolateral anastomosis, with blind closure of the duodenum) 24 h before 96% ethanol 1 ml/rat i.g. Treatment (/kg b.wt.) simultaneously with 96% ethanol (salutary agents: pentadecapeptide BPC 157 10 μg (μg) or 10 ng (ng), atropine 10 mg (A), ranitidine 10 mg (R), omeprazole 10 mg (O) i.p.; prostaglandins synthesis inhibitor indomethacin (IND) 5 mg s.c.). Controls received an equal volume of saline (5 ml/kg b.wt., i.p.). 10–15 rats per experimental group. $* P < 0.05$, at least vs. control.

protocols depart from the classic course. The variable effect of indomethacin now seen, no aggravation of final lesions in the endogenous/exogenous irritant contrary to other protocols, could suggest a novel aspect of the cytoprotection/adaptive cytoprotection issue: prostaglandin-dependent vs. prostaglandins-independent processes, exogenous vs. endogenous irritants), probably due to different preceding conditions—specially, induction of the endogenous irritants. Consistent with this, in the rats with gastrojejunal anastomosis, while these lesions could not be affected to any extent by indomethacin, a prostaglandin synthesis inhibitor, again both the pentadecapeptide BPC 157, and omeprazole further attenuated the strong ethanol-induced lesions, to below the control (surgery-endogenous irritants + strong ethanol) values, as they had done in exogenous/exogenous irritant-experiments. Thus, their beneficial effects could still be convincingly interpreted as augmentation of adaptive cytoprotective activity. Both, the 'reactivation' of pentadecapeptide BPC 157 and the reversal of omeprazole activity in the endogenous/exogenous irritant studies as compared to their effect with the endogenous irritant, and the change in indomethacin activity suggest that additional processes are activated after late challenge with strong ethanol in former experiments. The ineffectiveness of atropine is consistent with this suggestion. The potentiation by ranitidine was similar, and could suggest a role of the histamine system in cytoprotection/adaptive cytoprotection (Mozsik et al., 1995; Bodis et al., 1997).

Finally, while most lesions were in the glandular part, some appeared in the forestomach, where few if any ethanol lesions were seen in the intact rats. These lesions were reduced (or aggravated) along with most of the other lesions, if the lesions area was lessened, i.e., pentadecapeptide BPC 157, omeprazole, or augmented, i.e., ranitidine, by antiulcer agents application. This shows further that the events just described may be at least partly specific for endogenous/exogenous irritant conditions in rats with gastrojejunal anastomosis damaged by the strong-ethanol application.

3.5. The influence of no availability of food and water on ethanol-induced lesions

The possibility that the relative attenuation of lesions, i.e., adaptive cytoprotection, is a passive result dilution of the strong ethanol, or of buffering capacity of the gastric content with food remnants, was tested in animals with free access to food and water during the experiment. It was shown that animals with a stomach full of recently eaten food, had the same ethanol lesions as seen in the rats deprived of food and water before ethanol instillation (data not shown). There thus did not seem to have been an effect of ethanol dilution.

The general similarity of the results (not shown) with fed rats to all those obtained in food-withdrawn animals, regardless of irritant or surgery protocol eliminates dilution of the strong ethanol as a crucial factor in cytoprotection (Robert et al., 1974, 1983; Paré and Glavin, 1986; Holzer, 1991; Kato et al., 1996).

There is further evidence (from above) that the new model is suitable for its initial purpose: to split a seemingly homologous 'cytoprotection' activity into separate, and likely distinct agents effects.

Microscopic examination showed that in ethanol controls, necrotic areas involved the entire thickness of the mucosa. There was regenerating activity on the surface, but the epithelium was not restored. This extensive epithelial destruction was confirmed by transmission electron microscopy. Many superficial epithelial cells showed various stages of degeneration with derangement of organelles and excessive mucus shedding. In animals treated with BPC 157, the pattern of injury differed from that in controls. The surface epithelium showed less damage during the entire experiment and most lesions did not penetrate further than the upper part of the gastric glands. Less congestion and haemorrhage were also obvious. Some areas of deep necrosis and 'submucous ulcers' (representing necrotic areas covered by intact mucosa) were observed. Transmission electron microscopy also showed that, despite some degenerative changes in epithelial cells, the cohesion of the cells seemed much better preserved than in the controls. These results were also confirmed by scanning electron microscopy which, showed excessive basement membrane denudation in the controls, but not in the BPC 157 treated rats. The histological patterns seen for other drug regimens correlated fully with the data from visual/gross observation.

4. Discussion

The nature of the preceding exogenous small irritant effect (exogenous (exogenous irritant) was shown to differ from the endogenous one (endogenous irritant studies)). This difference modifies the activity of agents, initially thought to be simply 'cytoprotective'. Their effectiveness could be only simple-cytoprotective, or in a more complex way, adaptive–cytoprotective, or both cytoprotective and adaptive–cytoprotective activities, depending on the experimental conditions. For instance, in a seemingly homogenous group with respect to cytoprotection (Sikiric et al., 1997c), atropine and ranitidine have only cytoprotective activity (exogenous irritant experiments), whereas pentadecapeptide BPC157 and omeprazole have especially adaptive cytoprotective activity (endogenous/exogenous irritant experiments) or both cytoprotective and adaptive cytoprotective activities (exogenous/exogenous irritant experiments).

Primary minor lesions that appeared following a mild irritant, either exogenous (Robert et al., 1983), or endogenous (as described above) presented the basal lesion level at the moment of strong ethanol application, i.e., exogenous/exogenous irritant experiments vs. endogenous/exogenous irritant experiments. If challenged with strong irritant, i.e., strong ethanol, these lesions responded variously but did show that further attenuation was still possible. This was the first clear demonstration that adaptive cytoprotection can still function if the endogenous balance is disturbed.

By extending the application protocols (four points vs. one point), we provided evidence that the level of these lesions, minor and major, and the course of their development, could be modified by the antiulcer agents or by indomethacin (commonly used as a prostaglandin synthesis inhibitor, i.e., Robert, 1979; Robert et al., 1983; Robert, 1985; Robert et al., 1991) depending on the timing of application: (i) surgery, (ii) mild ethanol, (iii) strong ethanol, (iv) strong ethanol applied after a suitable period following either mild ethanol or surgery. The data thus obtained showed that the question of cytoprotection/adaptive cytoprotection involves considerable heterogeneity. Several models of reactions (prostaglandin-dependent vs. prostaglandin-independent, exogenous vs. endogenous small irritants) should thus be proposed. It is likely, that the models are sequentially involved, prior to or after challenge with a strong irritant. We propose that only the first cytoprotective part is prostaglandin-dependent in both models. The second, adaptive–cytoprotective part, i.e., after second challenge in the series, is strong ethanol administration, could be either prostaglandin-dependent (exogenous/exogenous irritant) or prostaglandin-non-dependent (endogenous/exogenous irritant). The involvement of other systems, i.e., cholinergic, histaminergic, peptidergic, is very likely, particularly in experiments with endogenous irritants. Admittedly, this suggestion that new model together with classic model is suitable for splitting a seemingly homologous 'cytoprotection'-activity into separate, and likely distinctive agents effects, is based on the results with indomethacin and antiulcer agents activity. However, this is also the case with the classic understanding of cytoprotection/adaptive cytoprotection (Robert, 1979; Robert et al., 1983; Moron et al., 1984; Robert, 1985; Szabo et al., 1985; Clissold and Campoli-Richards, 1986; Szabo, 1986, 1989; Robert et al., 1991; Sikiric et al., 1993b, 1994, 1995; Kato et al., 1996), whereas precise mechanism remains elusive.

Nevertheless, along with spontaneous lesions reduction, as an essential tool of adaptive cytoprotection (Robert et al., 1983; Robert, 1985), the further lesions reduction achieved with agents, such as pentadecapeptide BPC 157 (Sikiric et al., 1993a,b, 1994; Bosnjak et al., 1994; Paré and Kluczynski, 1994; Veljaca et al., 1994a,b, 1995a,b; Sikiric et al., 1995; Sandor et al., 1996; Sikiric et al., 1996b, 1997a,b,c,d,e; Seiwerth et al., 1997; Sandor et al., 1997; Bodis et al., 1997) or omeprazole (Clissold and Campoli-Richards, 1986), suggests that they are essential links between the various reactions in cytoprotection/adaptive cytoprotection chain.

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References

Bodis, B., Karadi, O., Nagy, L., Dohoczky, Cs., Kolega, M., Mozsik, Gy., 1997. Direct cellular effects of some mediators, hormones and growth factor-like agents on denervated (isolated) rat gastric mucosal cells. J. Physiol. (Paris) 91, 183–187.

Bosnjak, Z.J., Graf, B.M., Sikiric, P., 1994. Protective effects of newly isolated gastric peptide following hypoxic and reoxygenation injury in the isolated guinea pig heart. FASEB J. 8, A129.

Clissold, S.P., Campoli-Richards, D.M., 1986. Omeprazole: an updated review. Drugs 32, 15–47.

Erceg, D., Simicevic, V.N., Kolega, M., Dohoczky, C., 1997. Some aspects of PL-10.1.AK-15 on the gastrointestinal tract. J. Physiol. (Paris) 91, 179–181.

Holzer, P., 1991. Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. Rev. Physiol. Biochem. Pharmacol. 121, 49–146.

Kato, K., Yang, H., Tache, Y., 1996. Role of prostaglandins and calcitonin gene-related peptide in central vagal cholinergic dependent protection against gastric injury in urethane-anesthetized rats. Digestion 57, 322–327.

Moron, F., Pena, C., Cuesta, E., Mozsik, G., Javor, T., 1984. Comparative study of the cytoprotective effects of anticholinergic agents on the gastric mucosal lesions produced by intragastric administration of 0.6 M HCl in rats. In: Mózsik, G., Pár, A., Bertelli, A. (Eds.), Recent Advances in Gastrointestinal Cytoprotection. Akedémiai Kiado, Budapest, pp. 61–66.

Mozsik, Gy., Abdel-Salam, O.M.E., Suto, G., Kiraly, A., Vincze, A., Karadi, O., 1995. Acetylcholine, pentagastrin, histamine and 2,4-di-

nitrophenol stimulate the PGI2-induced gastric cytoprotection in ethanol-treated rats. Z. Gastroenterol. 5, 301.

Paré, W., Glavin, G., 1986. Restraint stress in biomedical research: a review. Neurosci. Biobehav. Rev. 10, 339–370.

Paré, W., Kluczynski, J.M., 1994. The effect of new gastric juice peptide BPC on classic stress triad in stress procedure. Exp. Clin. Gastroenterol. 2, 234–236.

Robert, A., 1979. Cytoptotection by prostaglandins. Gastroenterology 77, 761–767.

Robert, A., 1985. Cytoprotection and adaptive cytoprotection. In: Nelis, G.F., Boevé, J., Misiewicz, J.J. (Eds.), Peptic Ulcer Disease: Basic and Clinical Aspects. Martinus Nijhoff Publishers, Dordrecht, pp. 297–316.

Robert, A., Nezamis, J.E., Lancaster, C., Badalamenti, J.N., 1974. Cystamine-induced duodenal ulcers: a new model to test antiulcer agents. Digestion 11, 199–214.

Robert, A., Nezamis, J.E., Lancaster, C., Davis, J.P., Field, S.O., Hanchar, A.J., 1983. Mild irritants prevent gastric necrosis through 'adaptive cytoprotection' mediated by prostaglandins. Am. J. Physiol. 245, G113–G121.

Robert, A., Lancaster, C., Olafsson, A.S., Gilbertson-Beadling, S., Zhang, W., 1991. Gastric adaptation to the ulcerogenic effect of aspirin. Exp. Clin. Gastroenterol. 1, 73–81.

Sandor, Z., Vincze, A., Szabo, S., 1996. The protective effect of a recently isolated gastric peptide in acute and chronic gastroduodenal injury. FASEB J. 10, 171.

Sandor, Z., Vincze, A., Jadus, M.R., Dohocky, C., Erceg, D., Brajsa, K., Kolega, M., Szabo, S., 1997. The protective effect of newly isolated peptide PL-10 in the iodoacetamide colitis model in rats. Gastroenterology 112, 400.

Seiwerth, S., Sikiric, P., Grabarevic, Z., Zoricic, I., Hanzevacki, M., Ljubanovic, D., Coric, V., Konjevoda, P., Petek, M., Rucman, R., Turkovic, B., Perovic, D., Mikus, D., Jadrijevic, S., Medvidovic, M., Tadic, T., Romac, B., Kos, J., Peric, J., Kolega, Z., 1997. BPC 157 effect on healing. J. Physiol. (Paris) 91, 173–178.

Sikiric, P., Banic, M., Brkic, T., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Anic, B., Rotkvic, I., Duvnjak, M., Veljaca, M., Simicevic, V., 1993a. Effect of as novel pentadecapeptide BPC 157 and methylprednisolone in a murine model of inflammatory bowel disease. Gastroenterology 104, 782.

Sikiric, P., Petek, M., Rucman, R., Seiwerth, S., Grabarevic, Z., Rotkvic, I., Turkovic, B., Jagic, V., Mildner, B., Duvnjak, M., Lang, N., 1993b. A new gastric juice peptide BPC—an overview of stomach/stress/organoprotection hypothesis and BPC beneficial effects. J. Physiol. (Paris) 87, 313–327.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Petek, M., Rucman, R., Turkovic, B., Rotkvic, I., Jagic, V., Duvnjak, M., Mise, S., Djacic, S., Separovic, J., Veljaca, M., Sallmani, A., Banic, M., Brkic, T., 1994. The beneficial effect of BPC 157, a 15 aminoacid peptide BPC fragment, on gastric and duodenal lesions induced by restraint stress, cysteamine and 96% ethanol in rats. A comparative study with H2 receptor antagonists, dopamine promoters and gut peptides. Life Sci. 54, PL63–PL68.

Sikiric, P., Rotkvic, I., Mise, S., Seiwerth, S., Grabarevic, Z., Petek, M., Rucman, R., Zjacic-Rotkvic, V., Duvnjak, M., Jagic, V., Suchanek, E., Marovic, A., Banic, M., Brkic, T., Hanzevacki, M., Separovic, J., Djacic, S., Simicevic, V., 1995. Dopamine agents efficacy in peptic ulcer healing and relapse prevention—a further indication for importance of stomach dopamine in the stress organoprotection concept. In: Szabo, S., Taché, Y., Glavin, G. (Eds.), Neuroendocrinology of Gastrointestinal Ulceration: Hans Selye Symposia on Neuroendocrinology and Stress, Vol. 2. Plenum, New York, pp. 221–230.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Gjurasin, M., Konjevoda, P., Separovic, J., Ljubanovic, D., Artukovic, B., Bratulic, M., Tisljar, M., Jurina, Lj., Buljat, G., Miklic, P., Marovic, A., 1996b. The beneficial effect of a novel pentadecapeptide BPC 157 on gastric lesions induced by restraint stress, ethanol, indomethacin and capsaicin neurotoxicity. Dig. Dis. Sci. 41, 1604–1614.

Sikiric, P., Hanzevacki, M., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Mise, S., Rotkvic, I., Turkovic, B., Jagic, V., Separovic, J., Konjevoda, P., Petrov, G., Prkacin, I., 1997a. The effect of pentadecapeptide BPC 157, H2 blockers and sucralfate on new vessels and new granulation tissue formation. Gastroenterology 112, 291.

Sikiric, P., Mazul, B., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Jurina, Lj., Konjevoda, P., Hanzevacki, M., Gjurasin, M., Separovic, J., Ljubanovic, D., Artukovic, M., Bratulic, M., Tisljar, M., Miklic, P., Sumajstorcic, J., 1997b. Pentadecapeptide BPC 157 interactions with adrenergic and dopaminergic systems in mucosal protection in stress. Dig. Dis. Sci. 42, 661–671.

Sikiric, P., Mikus, D., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Peric, J., Konjevoda, P., Perovic, D., Jurina, L., Hanzevacki, M., Separovic, J., Gjurasin, M., Jadrijevic, S., Jelovac, N., Miklic, P., Buljat, G., Marovic, A., 1997c. Pentadecapeptide BPC 157, cimetidine, ranitidine, bromocriptine and atropine effect in cysteamine lesions in totally gastrectomized rats. A model study for cytoprotective studies. Dig. Dis. Sci. 42, 1029–1037.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Konjevoda, P., Perovic, D., Jurina, L., Separovic, J., Hanzevacki, M., Artukovic, B., Bratulic, M., Tisljar, M., Gjurasin, M., Miklic, P., Stancic-Rokotov, D., Slobodnjak, Z., Jelovac, N., Marovic, A., 1997d. The influence of a novel pentadecapeptide BPC 157 on L-NAME and L-arginine effect on stomach mucosal integrity and blood pressure. Eur. J. Pharmacol. 332, 23–33.

Sikiric, P., Seiwerth, S., Grabarevic, Z., Rucman, R., Petek, M., Jagic, V., Turkovic, B., Rotkvic, I., Mise, S., Zoricic, I., Perovic, D., Simicevic, V., Hanzevacki, M., Ljubanovic, D., Artukovic, M., Bratulic, M., Tisljar, M., Rekic, B., Gjurasin, M., Miklic, P., Buljat, G., 1997e. Pentadecapeptide BPC 157 positively affects both non-steroidal anti-inflammatory agents-induced gastrointestinal lesions and adjuvant arthritis in rats. J. Physiol. (Paris) 91, 113–122.

Szabo, S., 1986. Experimental basis for a role for sulfhydryls and dopamine in ulcerogenesis: a primer for cytoprotection–organoprotection. Klin. Wochenschr. 64, 116–122, (Suppl. 7).

Szabo, S., 1989. Critical and timely review of the concept of gastric cytoprotection. In: Mozsik, G., Javor, T., Kitajima, M., Pfeiffer, C.J., Rainsford, K.D., Simon, L., Szabo, S. (Eds.), Advances in Gastrointestinal Cytoprotection: Topics 1987. Akademiai Kiado, Budapest, pp. 115–127.

Szabo, S., Trier, J.S., Brown, A., Schnoor, J., 1985. Early vascular injury and increased vascular permeability in gastric mucosal injury caused by ethanol in the rat. Gastroenterology 88, 228–236.

Veljaca, M., Pllana, R., Lesch, C.A., Sanchez, B., Chan, K., Guglietta, A., 1994a. Protective effect of BPC 157 on a rat model of colitis. Gastroenterology 106, 789.

Veljaca, M., Lech, C.A., Pllana, R., Sanchez, B., Chan, K., Guglietta, A., 1994b. BPC-15 reduces trinitrobenzene sulfonic acid-induced colonic damage in rats. J. Pharmacol. Exp. Ther. 272, 417–422.

Veljaca, M., Chan, K., Guglietta, A., 1995a. Digestion of h-EGF, h-TGF alfa, and BPC 15 in human gastric juice. Gastroenterology 108, 761.

Veljaca, M., Lesch, C.A., Sanchez, B., Low, J., Guglietta, A., 1995b. Protection of BPC-15 on TNBS-induced colitis in rats: possible mechanisms of action. Gastroenterology 108, 936.