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European Journal of Pharmacology 477 (2003) 73–80
www.elsevier.com/locate/ejphar

Different effect of antiulcer agents on rat cysteamine-induced duodenal ulcer after sialoadenectomy, but not gastrectomy

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Received 23 January 2003; received in revised form 1 August 2003; accepted 8 August 2003

Abstract

The focus was on salivary glands in cysteamine-induced duodenal ulcer and the different effects of antiulcer agents on cysteamine-induced duodenal ulcer in sialoadenectomized but not gastrectomized rats. We tested antiulcer agents on cysteamine-induced duodenal ulcer in rats (agents/kg i.p.) simultaneously with cysteamine 400 mg/kg s.c., rat killed 24 h thereafter subjected to no surgery (normal), to gastrectomy (24 h before) or sialoadenectomy, acute (24 h before) or chronic (21 days before). (i) Ulcerogenesis: cysteamine-induced duodenal ulcer had the same severity and incidence in normal, gastrectomized or acutely or chronically sialoadenectomized rats. (ii) Antiulcer effect under normal conditions or following gastrectomy: in normal or gastrectomized rats all agents tested, gastric pentadecapeptide BPC 157 [currently in clinical trials for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva) (10.0 μg or 10.0 ng), ranitidine (10 mg), atropine (10 mg), omeprazole (10 mg)] inhibited cysteamine-induced duodenal ulcers, acting through gastric acid-independent mechanisms. Following sialoadenectomy, acute or chronic: ranitidine, omeprazole and atropine were completely ineffective, while pentadecapeptide BPC 157 could protect. Thus, we found that contrary to stomach, salivary glands are implicated in cytoprotective agent activity (standard agents were ineffective after sialoadenectomy). Also, gastric pentadecapeptide BPC 157 was consistently associated with a cytoprotective effect, suggesting a beneficial activity distinctive from that of H2-receptor blockers, proton-pump inhibitors and anticholinergics; but probably replacing missing salivary glands factors.

Keywords: Salivary gland; Cysteamine; Duodenal ulcer; Gastrectomy; Sialoadenectomy; Cytoprotection; Gastric pentadecapeptide BPC 157 (PLD-116); Ranitidine; Atropine; Omeprazole

1. Introduction

Long implicated in the maintenance of gastrointestinal tract mucosal integrity, the salivary glands and thus effects of sialoadenectomy have been investigated in many, mostly gastric ulcer models (e.g., Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995). In contrast, cysteamine-induced duodenal ulcer-related pathology (mitigated by EGF-addition) has been addressed only in one study (Kunizaki et al., 1989), while neither cysteamine-induced duodenal ulcers severity nor antiulcer therapy effect was specifically investigated in acutely or chronically sialoadenectomized animals. This may be relevant since, as developed by Selye and Szabo (1973), Szabo (1979) and Szabo and Neumeyer (1983), cysteamine-induced duodenal ulcer remains one of the most important models for evaluating the effect of antiulcer agents effect (e.g., Selye and Szabo, 1973; Szabo, 1979; Szabo and Neumeyer, 1983; Sikiric et al., 1997a, 2001a,b; Sandor et al., 1996). A point that needs to be investigated is whether the removal of salivary glands would eliminate a significant endogenous source of growth factors, and minimize or eliminate their otherwise important influence on healing (e.g., Konturek et al., 1991a,b). This would likely lead to exaggerated cysteamine-duodenal ulcer severity, and weakened inhibition of cysteamine damage by antiulcer agents. Another important point is that a cysteamine-in-

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0014-2999/$ - see front matter © 2003 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2003.08.013

duced duodenal ulcer now appears to be relevant in cytoprotection research (i.e., complete elimination of gastric acid as ulcer-inducer). A cysteamine-induced duodenal ulcer in gastrectomized rats, inhibited by all antiulcer agents tested (Sikiric et al., 1997a), would appear to be an essential prerequisite for a pure cytoprotective study.

Previously, the existence of a cytoprotective effect, under gastric acid non-dependent conditions (Robert, 1979), could not be demonstrated without gastrectomy. Thus, this new cytotoxic/cytoprotective aspect involving cysteamine-induced duodenal ulcer (Sikiric et al., 1997a) merits further investigation, particularly the similarity of beneficial effect of antiulcer agents in gastrectomized rats. However, the contribution of other structures remains to be further investigated, including the involvement of salivary glands in cytoprotective agents' activity. Therefore, the inhibition of cysteamine-induced duodenal ulcer by antiulcer agents previously protecting both normal and gastrectomized rats (Sikiric et al., 1997a), such as gastric pentadecapeptide BPC 157, currently in clinical trials for inflammatory bowel disease (PL-10, PLD-116, PL-14736, Pliva), ranitidine, omeprazole and atropine, was further investigated in cysteamine-challenged rats not subjected to surgery, whether gastrectomy or sialoadenectomy, acute or chronic. Results of this research may shed light on salivary glands functions. Mostly, their functioning after elimination of salivary glands provided a basis for understanding the action of so far identified factors (i.e., EGF, NGF) (Matsuda et al., 1998; Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995), calcitonin-gene related peptide (CGRP) (Evangelista et al., 1991), substance P, vasoactive intestinal peptide (VIP) (for review, see Walsh and Dockray, 1994).

2. Methods

2.1. Animals

Wistar female Albino rats randomly assigned, 10–16 rats per group, weighing 200–220 g b.w., were used in all experiments.

2.2. Drugs and drugs application

Pentadecapeptide BPC157 (GEPPPGKPADDAGLV, M.W. 1419) (manufactured by Diagen, d.o.o., Ljubljana, Slovenia) is a partial of sequence of human gastric juice protein BPC, freely soluble in water at pH 7.0 and in saline. It was prepared as described earlier. Peptide with 99% (HPLC)

Table 1
The effect of gastrectomy and sialoadenectomy, acute or chronic on cysteamine-duodenal lesion incidence

Surgical protocol before cysteamine (400 mg/kg s.c.)	Agent (/kg i.p.) simultaneously with cysteamine (400 mg/kg s.c.)	Cysteamine-rats		Two-tailed Fisher's exact probability test (p)
		Number of rats with duodenal lesion	Number of rats without duodenal lesion	
None	Saline	10	0	
	Ranitidine 10 mg	2	8	0.0007
	Omeprazole 10 mg	1	9	0.0001
	Atropine 10 mg	2	8	0.0007
	BPC157 10 μg	0	10	0.0000
	BPC157 10 ng	0	10	0.0000
Gastrectomy	Saline	10	0	
	Ranitidine 10 mg	2	8	0.0007
	Omeprazole 10 mg	1	9	0.0001
	Atropine 10 mg	2	8	0.0007
	BPC157 10 μg	0	10	0.0000
	BPC157 10 ng	0	10	0.0000
Sialoadenectomy, acute	Saline	10	0	
	Ranitidine 10 mg	10	0	N.S.
	Omeprazole 10 mg	10	0	N.S.
	Atropine 10 mg	10	0	N.S.
	BPC157 10 μg	0	10	0.0000
	BPC157 10 ng	0	10	0.0000
Sialoadenectomy, chronic	Saline	10	0	
	Ranitidine 10 mg	10	0	N.S.
	Omeprazole 10 mg	10	0	N.S.
	Atropine 10 mg	10	0	N.S.
	BPC157 10 μg	2	8	0.0007
	BPC157 10 ng	3	7	0.0031

At 24 h (gastrectomy, sialoadenectomy) or 21 days (sialoadenectomy), rats received cysteamine (400 mg/kg s.c.) simultaneously with an intraperitoneal administration of gastric pentadecapeptide BPC 157, ranitidine, omeprazole, atropine or saline. Assessment at 24 h following cysteamine. Comparison vs. control (saline 5.0 ml/kg i.p.) in each protocol group (two-tailed Fisher's exact probability test).

purity (1-des-Gly peptide as impurity), dissolved in saline was used in all experiments (Sikiric et al., 1997a,b, 1999a,b,c, 2001a,b, 2003; Mikus et al., 2001; Sebecic et al., 1999).

Simultaneously with cysteamine, the animals received (i.p./kg b.w.) pentadecapeptide BPC 157 or various other agents (dissolved in saline, except for omeprazole (prepared as before; Sikiric et al., 1997a, 1999a,b), using doses shown to effective in previous studies (Mikus et al., 2001; Sikiric et al., 1997a, 1999a,b) BPC 157 (10.0 μg or 10.0 ng), ranitidine (10 mg; Ranital, Pliva, Croatia), omeprazole (10 mg; Ultop, Krka, Slovenia), atropine (10 mg, atropine sulphate; Sigma, USA) or an equal volume of saline (5 ml).

2.3. Experimental designs

2.3.1. Sialoadenectomy/gastrectomy/cysteamine application

Total gastrectomy or only laparatomy was carried out under ether anaesthesia. After removal of the stomach, the esophagus and duodenum were joined by a termino-terminal anastomosis. Ether anaesthesia was also used for sialoadenectomy or sham operation. Then, to establish the effect of surgery, i.e., at 24-h post-surgery (acute sialoadenectomy; gastrectomy) or at 21-day post-surgery (chronic sialoadenectomy), some of the animals were killed before cysteamine application. Other rats received cysteamine (cysteamine–HCl, Sigma) (400 mg/kg s.c. dissolved in distilled water) and were killed 24 h after cysteamine application, as described before (Sikiric et al., 1997a). Animals operated, but not treated with cysteamine following surgery, were killed at the end of a 48-h or a 22-day postoperative period.

2.3.2. Assessment of mucosal injury

Immediately after killing, the duodenum was removed and lesions severity (sum of longest diameters of lesions; mm) and incidence (the number of rats with ulcer or without ulcer) were assessed by naive observers as described before (Mikus et al., 2001; Sikiric et al., 1994, 1997a, 1999a,b, 2001a,b, 2003). Representative tissue sections were processed for further histologic analysis.

2.4. Statistical analysis

Statistical analysis was performed using a nonparametric Kruskal–Wallis analysis of variance (ANOVA) and subsequent Mann–Whitney *U*-test to compare groups. Using Bonferroni's correction, values of $P < 0.008$ were considered statistically significant in the Mann–Whitney *U*-test when six treatment groups were compared. Percentages of rats with and without gastric ulcers were compared using a two-tailed Fisher's exact probability test. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Cysteamine-duodenal ulcers in intact rats

As expected, given alone, cysteamine produces macroscopic duodenal ulcers. These ulcers (both ulcer incidence, a decreased percentage of rats with duodenal ulcers, and ulcer size) were markedly reduced by all antiulcer agents given at their doses effective in previous studies (Table 1, Fig. 1).

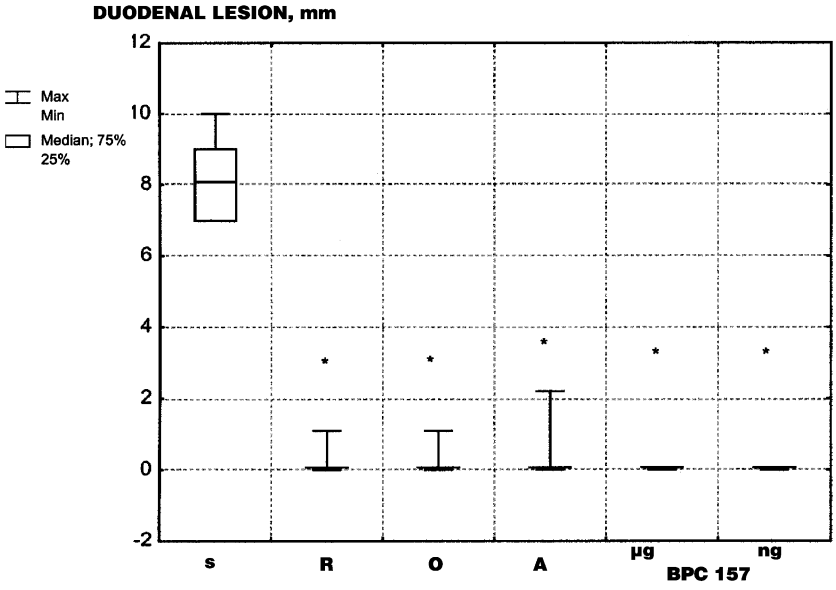

Fig. 1. Cysteamine-duodenal lesion severity (sum of longest lesions diameters; mm) in intact rats. Rats received cysteamine (400 mg/kg s.c.) simultaneously with an intraperitoneal administration of gastric pentadecapeptide BPC 157 (10.0 μg or 10.0 ng/kg) (μg, ng), ranitidine (10 mg/kg) (R), omeprazole (10 mg/kg) (O), atropine (10 mg/kg) (A) or an equivolume of saline (5 ml/kg) (s). Assessment at 24 h following cysteamine. Nonparametric Kruskal–Wallis ANOVA and subsequent Mann–Whitney *U*-test to compare groups. Due to Bonferroni's correction, values of $p < 0.008$ were considered statistically significant in Mann–Whitney *U*-test when six treatment groups were compared. *$p = 0.000157$ vs. control (s).

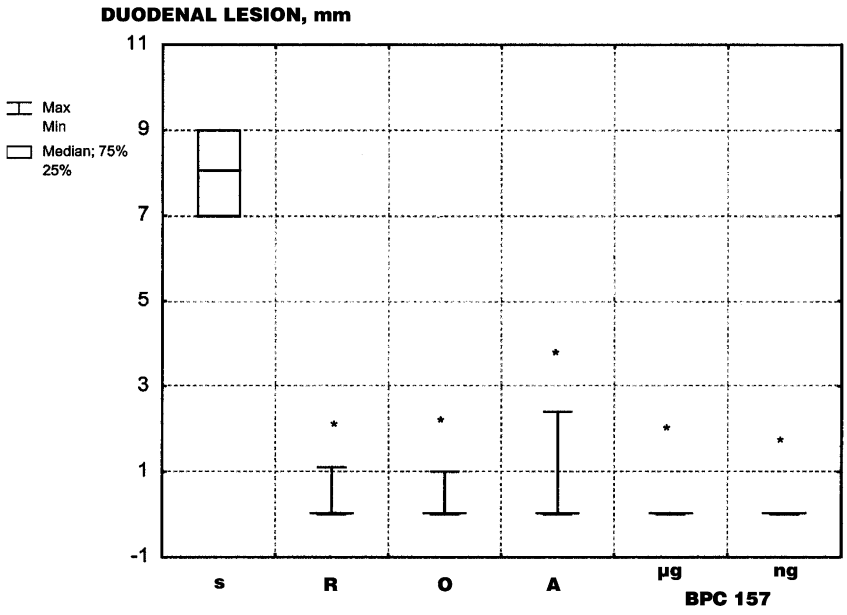

Fig. 2. Cysteamine-duodenal lesion severity (sum of longest lesions diameters; mm) in gastrectomized rats. At 24 h following gastrectomy, rats receive cysteamine (400 mg/kg s.c.) simultaneously with an intraperitoneal administration of gastric pentadecapeptide BPC 157 (10.0 µg or 10.0 ng/kg) (µg, ng), ranitidine (10 mg/kg) (R), omeprazole (10 mg/kg) (O), atropine (10 mg/kg) (A) or an equivolume of saline (5 ml/kg) (s). Assessment at 24 h following cysteamine. Nonparametric Kruskal–Wallis ANOVA and subsequent Mann–Whitney *U*-test to compare groups. Due to Bonferroni's correction, values of $p < 0.008$ were considered statistically significant in Mann–Whitney *U*-test when six treatment groups were compared. *$p = 0.000157$ vs. control (s).

3.2. Gastrectomy and cysteamine-duodenal ulcers

Under gastric acid-free conditions, i.e., in gastrectomized rats, the ulcerogenic effect of cysteamine, and protective effect of antiulcer agents, did not differ from the effect in normal rats (Table 1, Fig. 2). Cysteamine produced ulcers that are consistently inhibited by all antiulcer agents used (pentadecapeptide BPC157, ranitidine, omeprazole, and atropine).

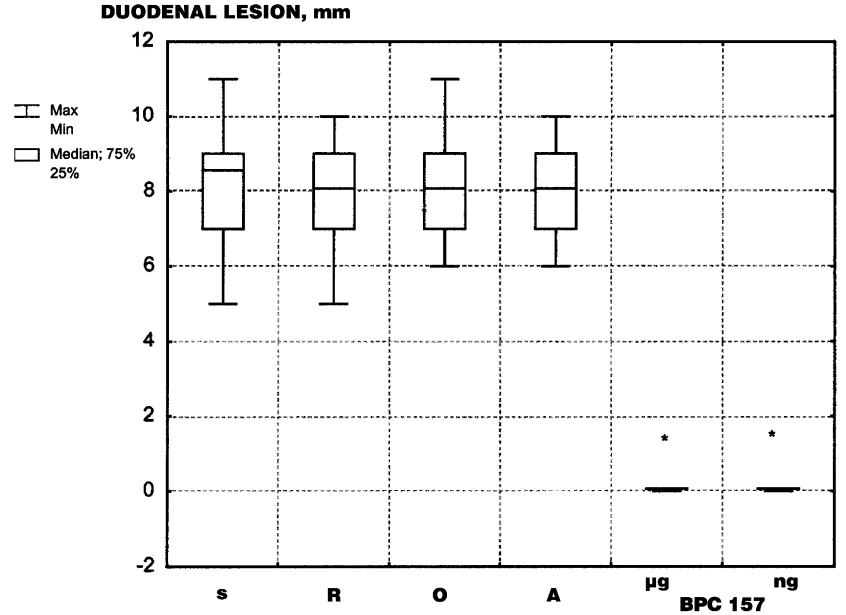

Fig. 3. Cysteamine-duodenal lesion severity (sum of longest lesions diameters; mm) in acutely sialoadenectomized rats. At 24 h following sialoadenectomy, rats received cysteamine (400 mg/kg s.c.) simultaneously with an intraperitoneal administration of gastric pentadecapeptide BPC 157 (10.0 µg or 10.0 ng/kg) (µg, ng), ranitidine (10 mg/kg) (R), omeprazole (10 mg/kg) (O), atropine (10 mg/kg) (A) or an equivolume of saline (5 ml/kg) (s). Assessment at 24 h following cysteamine. Nonparametric Kruskal–Wallis ANOVA and subsequent Mann–Whitney *U*-test to compare groups. Due to Bonferroni's correction, values of $p < 0.008$ were considered statistically significant in Mann–Whitney *U*-test when six treatment groups were compared. *$p = 0.000157$ vs. control (s).

3.3. Sialoadenectomy and cysteamine-duodenal ulcers

3.3.1. Acute sialoadenectomy

Following acute sialoadenectomy (24 h), the parameter for cysteamine-induced duodenal mucosal lesions did not increase over the values for normal rats. However, in acutely sialoadenectomized rats, the effect of antiulcer agents on cysteamine-induced duodenal ulcer became different. Ranitidine, omeprazole and atropine were completely ineffective (Table 1, Fig. 3). On the other hand, pentadecapeptide BPC 157 (both 10.0-μg and 10.0-ng doses) protected acutely sialoadenectomized rats against cysteamine-induced duodenal ulcer.

3.3.2. Chronic sialoadenectomy

Similar effects were seen after a more prolonged period (21 day) with cysteamine challenge, 21 days after sialoadenectomy, cysteamine-induced duodenal mucosal lesions showed no spontaneous increase in controls over values noted in normal rats. Again, ranitidine, omeprazole and atropine were completely ineffective, and gastric pentadecapeptide BPC 157 was effective with either the 10.0-μg or 10.0-ng regimen (Table 1, Fig. 4).

No duodenal lesion was present in sham-groups (gastrectomy, sialoadenoctomy, laparatomy, or submandibular skin incision) not treated with cysteamine. If laparatomy, or submandibular skin incision groups were treated with cysteamine, or cysteamine + antiulcer agents, the incidence and severity of duodenal lesions were the same as in the corresponding cysteamine control rats (non-sialoadenectomized, non-gastrectomized, nor subjected to laparatomy or submandibular skin incision). These data are not shown. Microscopically, mild mastocytic and moderate eosinophilic and mononuclear mucosal and submucosal infiltration were noted. These were less when the lesions were the clearly reduced.

4. Discussion

Evidence is provided from studies with cysteamine—that antiulcer agents exert their inhibitory effect on duodenal ulcer incidence and severity at least partly, through salivary glands. In view of its effectiveness also after sialoadenectomy, gastric pentadecapeptide BPC 157 was suggested as an additional factor for salivary gland healing functions, which may be important and have practical implication.

Extensively investigated, cysteamine-induced duodenal ulcer severity (Selye and Szabo, 1973; Szabo, 1979; Szabo and Neumeyer, 1983; Sikiric et al., 1997a, 2001a,b), and sialoadenectomy (e.g., Matsuda et al., 1998; Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995) had not be studied in combination. It still has been little or not investigated how salivary glands are related to cysteamine-induced duodenal ulcer severity, or how sialoadenectomy affects antiulcer agent inhibition of cysteamine-duodenal ulcer, having been

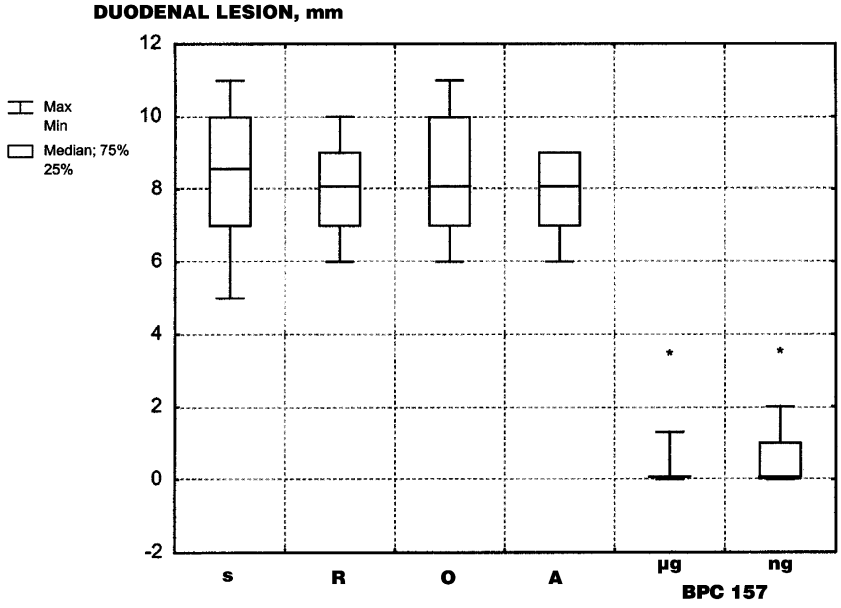

Fig. 4. Cysteamine-duodenal lesion severity (sum of longest lesions diameters; mm) in chronically sialoadenectomized rats. Following 21 days after sialoadenectomy, rats received cysteamine (400 mg/kg s.c.) simultaneously with an intraperitoneal administration of gastric pentadecapeptide BPC 157 (10.0 μg or 10.0 ng/kg) (μg, ng), ranitidine (10 mg/kg) (R), omeprazole (10 mg/kg) (O), atropine (10 mg/kg) (A) or an equal volume of saline (5 ml/kg) (s). Assessment at 24 h following cysteamine. Nonparametric Kruskal–Wallis ANOVA and subsequent Mann–Whitney *U*-test to compare groups. Due to Bonferroni's correction, values of $p < 0.008$ were considered statistically significant in Mann–Whitney *U*-test when six treatment groups were compared. *$p = 0.000157$ vs. control (s).

recognized as cytoprotective in experiments with gastrectomy (Sikiric et al., 1997a). Thus, salivary glands could be implicated in cysteamine-induced ulcer development or in cytoprotective antiulcer agent activity or both.

Post-gastrectomy cytoprotective activity of antiulcer agents may be a multifaceted phenomenon since these antiulcer agents influence several different systems (i.e., histaminergic, cholinergic, proton-pump function, peptidergic). Indeed, various antiulcer agents either had different effects or no effect on cysteamine-induced duodenal ulcer in sialoadenectomized rats (inhibition of cysteamine-induced ulcer with a particular agent disappears, i.e., ranitidine, atropine, omeprazole or still appears, i.e., gastric pentadecapeptide BPC157). Thus, this is the first time that there is direct evidence for the role of salivary glands in cytoprotective effects of these antiulcer agents, which had been evidenced indirectly, since cysteamine-induced duodenal ulcers are not affected by sialoadenectomy. Contrary, the same evidence does not implicate salivary glands in cysteamine-induced duodenal ulcer development. Potentiation would be expected. It is known that sialoadenectomy alone increases ethanol-, indomethacin-, or stress-induced gastric mucosal lesions (Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995).

With respect to therapy outcome, the sialoadenectomy effect was commonly referred as being salivary gland-specific, based on effects of replacement after sialoadenectomy (i.e., by EGF addition) (Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995). Previously, in sialoadenectomized rats, the development of cysteamine-induced gastric ulcers was counteracted by addition of EGF (Olsen et al., 1984) and cysteamine-induced duodenal ulcer-related pathology was mitigated by EGF-addition (although cysteamine-induced ulcer size and incidence were not specifically investigated) (Kunizaki et al., 1989). Therefore, ranitidine, omeprazole and atropine were completely inactive after both acute and chronic sialoadenectomy, while they remained active after gastrectomy, suggesting that H2-receptor blockers, anticholinergics and proton pump inhibitors collaborate closely in duodenal mucosa protection with factors from salivary gland, but not from stomach. This failure of agent effectiveness also suggests that histamine H_2-receptor antagonist, anticholinergic, proton pump inhibitor by themselves could not replace the salivary gland factor(s) necessary for cytoprotection maintenance. Alternatively, and more importantly, a consistent protective effect of gastric pentadecapeptide BPC 157 to counteract cysteamine-induced duodenal ulcer in healthy, gastrectomized and, particularly, sialoadenectomized rats, distinguishes its activity from that of H2-receptor blockers, proton-pump inhibitors and anticholinergics. Possibly, in analogy with results of EGF studies (Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995), the factors from salivary glands that are lacking in sialoadenectomized rats otherwise leading to failure of standard antiulcer agent activity, are suitably replaced only by pentadecapeptide BPC 157 application. The fact that both (10.0 μg and 10.0 ng) regimens are effective, both after short (acutely sialoadenectomized rats) and more prolonged (i.e., weeks) insufficiency (chronically sialoadenectomized rats) support this possibility. Also, removal of salivary glands to eliminate the endogenous source of EGF does not affect sucralfate-induced gastroprotection (Konturek et al., 1991a). Both gastric pentadecapeptide BPC 157 and sucralfate lead to angiogenesis and inflammatory tissue production (Sikiric et al., 1999c), essential for better ulcer healing and tissue repair (Szabo et al., 1985). For example, gastric pentadecapeptide BPC 157 induces strong angiogenesis even in hypocellular, hypovascular and hyponeural tissue (i.e. Achilles tendon) (Staresinic et al., in press).

Besides this, the findings in sialoadenectomized and gastrectomized rats, combined together, further support specific effect related to a lack of factors from salivary glands in sialoadenectomized rats. If nonspecific, more severe conditions had to debilitate gastrectomized rats, but they were protected with all given agents. Namely, besides gastrointestinal tract function, either sialoadenectomy or gastrectomy disturbs functioning of many systems: i.e., liver (Lambotte et al., 1997), skin (Inaloz et al., 2000), bone (Kobayashi et al., 1994; Sebecic et al., 1999), etc. However, at the time of cysteamine application, only gastrectomy produces major trauma, and results in a poor general condition (Sikiric et al., 1997a). Thus, the failure of ranitidine, atropine and omeprazole to protect, we now found in sialoadenectomized rats (but not in gastrectomized), is likely to be specific for a sialoadenectomy-induced condition (regardless an essential no specificity due to sialoadenectomy induced deficiency of EGF, NGF and other factors) (Evangelista et al., 1991; Walsh and Dockray, 1994). Besides, gastric pentadecapeptide BPC 157, the only antiulcer agent effective in normal, gastrectomized and sialoadenectomized rats, shows a more prominent cytoprotective activity (Sikiric et al., 1999b). It also strongly counters esophagitis development after gastrectomy in rats with esophagojejunal termino-lateral anastomosis (Sikiric et al., 1999a) with an effect more prominent than those of ranitidine or sucralfate.

Recently, in pharmacokinetic studies, gastric pentadecapeptide BPC 157 was also found in salivary glands (Veljaca et al., 2002). Therefore, it may be that pentadecapeptide BPC 157 is also responsible for salivary gland functions, in addition to factors so far identified (i.e., EGF, NGF) (Matsuda et al., 1998; Konturek et al., 1991a,b; Kohut et al., 1992; Hui et al., 1993; Wu et al., 1993; Tripp and Tepperman, 1995). For instance, in rats challenged with ethanol, sialoadenectomy augments the responsiveness of the gastric mucosa to NO administration (Tripp and Tepperman, 1995). Both the extent of mucosal damage and inhibition of NO synthase activity are exacerbated in sia-

loadenectomized rats. EGF administration reduces mucosal damage but does not restore NO synthase activity in ethanol-treated sialoadenectomized rats. Thus, EGF does not appear to influence mucosal NO synthase in ethanol-treated rats (Tripp and Tepperman, 1995). In contrast, pentadecapeptide BPC 157 strongly reverses the aggravated gastric lesions appeared after NOS-blockade (Sikiric et al., 1997b). Regarding the effect in gastric mucosa, it induces NO synthesis in gastric mucosal supernatant (Sikiric et al., 1997b). It is noteworthy that peptide stability is important for pathophysiology. Unlike EGF which is rapidly destroyed in human gastric juice, gastric pentadecapeptide BPC157 is stable for longer than 24 h in human gastric juice (Veljaca et al., 1995; Sikiric et al., 1997a,b, 1999a,b,c, 2001a,b, 2003; Mikus et al., 2001; Sebecic et al., 1999). Likewise, unlike NGF or EGF (Kiyohara et al., 1991; Liao et al., 2001), pentadecapeptide BPC157 does not require a carrier for its activity (Sikiric et al., 1997a,b, 1999a,b,c, 2001a,b, 2003; Mikus et al., 2001; Sebecic et al., 1999; Sandor et al., 1996; Staresinic et al., in press). Thus, pentadecapeptide BPC157 acts as a stable peptide, with easy delivery without carrier, unusual stability, and high resistance to otherwise highly degrading media. The special structure–acitivity relation(s) of pentadecapeptide BPC 157 outlined in our studies may contribute to avoiding the use of carrier(s) (Stambuk and Konjevoda, 1999; Mikus et al., 2001; Sikiric et al., 2003): pentadecapeptide BPC 157 lacks 11 amino acids shared by EGF family members, and six cysteine residues joined by three disulfide bonds (Walsh and Dockray, 1994). Various carriers for EGF and other peptides, with an effect of peptide + carrier(s) complex may yield results that are difficult to interpret (e.g., Urist, 1996), while not avoiding limited and uncertain active peptide delivery, and being inappropriate for routine use (Sanders and Hendren, 1997).

In summary, antiulcer agents ranitidine, atropine, and omeprazole inhibited duodenal ulcer incidence and severity, at least partly, through salivary glands in the rat. Unrecognized dysfunction of salivary glands as a cause of failed duodenal ulcer therapy in patients should be further tested. Finally, gastric pentadecapeptide BPC157, in clinical trials for inflammatory bowel disease (PL-10, PLD-116), also suggested to be responsible for salivary gland functions, promotes internal and external wound healing (Sikiric et al., 1994, 1997a,b, 1999a,b,c, 2001a,b, 2003; Mikus et al., 2001; Sebecic et al., 1999; Staresinic et al., in press) particularly when healing is impaired (i.e., corticosteroid-application; Sikiric et al., 2003), and in hypocellular, hypovascular and hyponeural tissue (i.e. Achilles tendon; Staresinic et al., in press).

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