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INTERLEUKIN-1 AND EXPERIMENTAL GASTRIC ULCER HEALING IN THE RAT

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The involvement of inflammation in peptic ulcer development and healing attracts growing interest. Since lymphokines, in particular interleukin-1 (IL-1), as ubiquitous mediators of inflammation are currently intensively studied in the gastrointestinal tract, we assessed the effect of this cytokine as well as that of a specific IL-1 release inhibitor (IX 207-887 (IX)) on development and healing of experimental gastric ulcers. After a single dose of IL-1, 4 $\mu\text{g}/\text{kg}$, i. p., basal acid secretion was almost completely inhibited for 4 hours in conscious chronic gastric fistula rats. In a first study, following induction of a 7 mm wide cryo-ulcer in the gastric corpus, three groups of 24 rats were treated either with a non-acid inhibitory dose of IL-1 (0.4 $\mu\text{g}/\text{kg}$) or with an antisecretory regimen (4 $\mu\text{g}/\text{kg}$) b. i. d. or saline control. Ulcer size did not differ from that of control animals, neither after 24h nor 7 days. Similarly, IX applied daily (20 mg/kg/s.c) from 5 days before ulcer induction and continued thereafter for 15 days had no effect on ulcer development or healing. Despite its anti-inflammatory property IX produced no macroscopically visible damage on the gastric or intestinal mucosa and may therefore offer a higher safety profile within the gastrointestinal tract than conventional non-steroidal anti-inflammatory drugs.

Key words: *Inflammation, interleukin-1, peptic ulcer healing.*

INTRODUCTION

The acute inflammatory response is essential to the healing process of gastrointestinal mucosal lesions (1). Mediators of the inflammatory reaction such as cytokines and their antagonists therefore have the theoretical potential to positively or negatively influence the development and healing of ulcerative processes in the gastrointestinal tract. Such an inference is well documented for inflammatory lesions in the colon and is particularly well established for interleukin-1 (2). This substance also has a potential for peptic ulcer healing, since this particular cytokine enhances the synthesis of prostaglandins which are linked to gastric mucosal protection (3,4) but does not necessarily

accelerate ulcer healing (5). Similar to prostaglandins IL-1 has gastroprotective properties against NSAID-mediated gastropathy (6, 7) and at higher doses can inhibit acetylcholine release in the rat myenteric plexus (8). Furthermore, it suppresses gastric acid secretion either stimulated with gastrin or histamine (3, 9—11). Such actions could be of physiologic significance since IL-1, in both of its major forms IL-1-alpha and -beta, is present in the normal gastric mucosa (12). These considerations prompted us to investigate whether IL-1 would have a potential beyond its ability to enhance prostaglandin synthesis, to influence the development and healing of gastric cryoulcers in a well established rat ulcer model (5, 13). The investigations were supplemented by similar studies with a recently developed IL-1 inhibitor, IX 207-887 (IX). This substance inhibits the release of both, IL-1-alpha and -beta and is currently under investigation for the treatment of chronic polyarthritis (14) where it achieved promising results in the first clinical trials (15).

MATERIALS AND METHODS

Pilot considerations

Establishment of an acid inhibitory dose of IL-1 in conscious chronic gastric fistula rats: Following intraperitoneal injection with different doses of IL-1, gastric juice pH was measured at regular intervals for eight hours. 4 $\mu\text{g}/\text{kg}$ resulted in a temporary near total suppression of intragastric acidity. IX 207-887 was given in a dose twice that which is effective in experimental arthritis and paw edema in the rat (14).

Study design (Table 1)

Table 1. Experimental design, 8 animals per group

[* = ulcer]	STUDY I, IL-1	STUDY II, IX—207—887	
	*7 d treatment	5 d pretreatment	* 14 d
treatment group 1	IL-1 4 $\mu\text{g}/\text{kg}$	IX 207—887	IX 207—887
group 2	IL-1 0.4 $\mu\text{g}/\text{kg}$	saline	IX 207—887
group 3	saline	saline	saline

STUDY I: Ulcer healing was studied in *three groups of 16* female Wistar rats, body weight 220—250g. Following ulcer induction the animals were treated for seven days with IL-1 0.4 $\mu\text{g}/\text{kg}$ (group 1), 4 $\mu\text{g}/\text{kg}$ (group II), or saline (group III) i. p., b. i. d. Macroscopic ulcer size was evaluated at days 1 and 7.

STUDY II: Five days prior to ulcer induction, *three groups of rats* were pretreated by gavage either with IX-207-887, 20 mg/kg/day/sc/ main (group I) or saline (groups II and III). Ulcers were created on day 0 and treatment was continued for 2 weeks with either IX-207-887 (groups I and II) or saline 1 ml/kg (group III).

Eight animals of each group were sacrificed day 1, 7 and 15 for assessment of macroscopic ulcer size.

During the entire study period the rats were kept under normal laboratory conditions with free access to water and a standard rat chow (Naphag, Gossau, Switzerland).

Experimental ulcers

Under general ether anaesthesia the abdomen was opened by median laparotomy. Using a technique previously described (13) and established in our laboratory (5), a cryo-injury was induced at the serosal surface of the anterior wall of the mid-corpus by application of a cryoprobe (0 6.5 mm, -60°C) for 45 seconds (Cryoprobe BM 250, Erbokryo 12, Rüeegg Medical, Baden, Switzerland).

Stomach preparation

Animals were sacrificed by an overdose of ether and incision of the left heart ventricle. The stomach was removed, opened along the greater curvature, rinsed with cold saline, pinned on a silicon layer and covered with 4% paraformaldehyde in a 0.04 M potassium phosphate buffer, pH 7.4. After 24 hours, the specimens were photographed and the macroscopically visible ulcer area was measured using a microprocessor-linked planimeter (Hipad Digitizer, Houston Instrument, Austin, Tx). Stomachs were also scored for mucosal damage such as erosions or haemorrhage, evaluating the number and size of haemorrhagic lesions.

In all evaluations the observer was neither aware of the treatment nor of the time of animal sacrifice.

Drugs

IX-207-887 was kindly provided by Sandoz AG, Basel, IL-1 alpha by Hoffmann La Roche, Nutley, New Jersey, USA.

Statistics

The significance of differences was determined by one way analysis of variance (ANOVA), with p values < 0.05 regarded as significant. Results are expressed as mean \pm SEM from 8 animals unless indicated otherwise.

RESULTS

Effect of IL-1 on gastric acidity

Intraperitoneal injection of IL-1 resulted in a dose dependent elevation of intragastric pH. This experiment demonstrates that $0.4\mu\text{g}/\text{kg}$ was

a non-antisecretory dose, while with $4\mu\text{g}/\text{kg}$ a near complete suppression of intragastric acidity was induced, four hours after injection. Basal values were regained after 6 hours (*Fig. 1*).

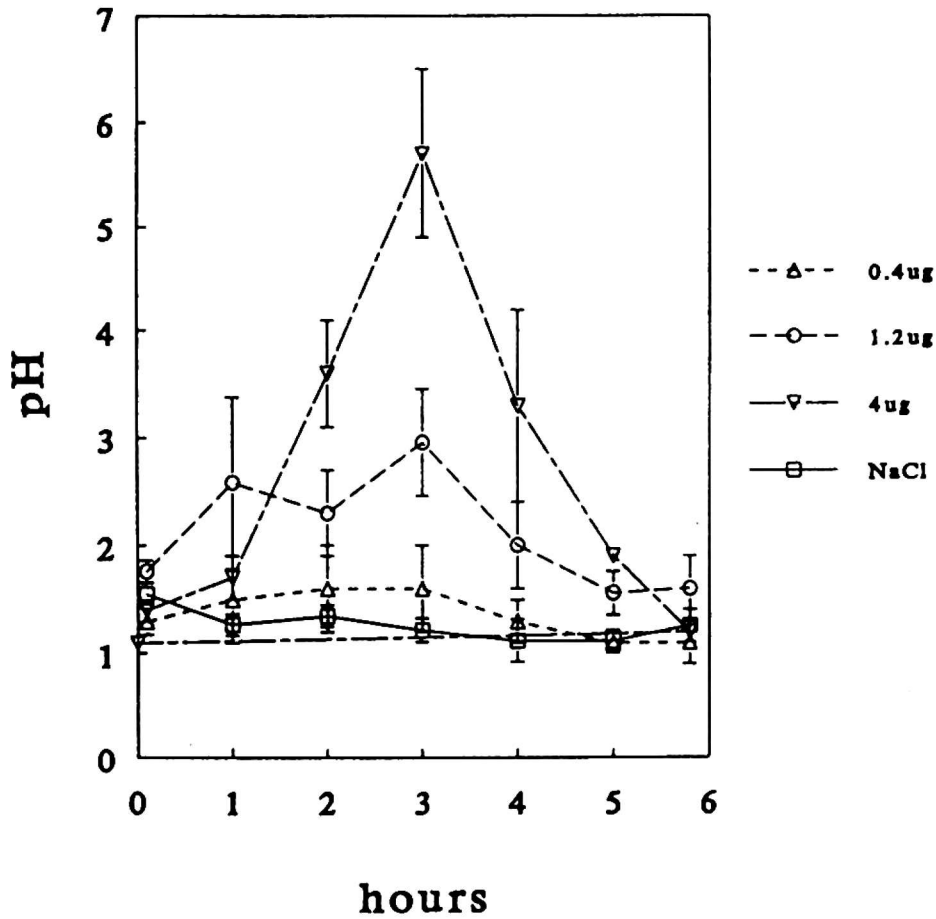


Fig. 1. Acid inhibition following single doses of IL-1. Gastric juice pH, mean + SEM of 6 animals. Doses of IL-1 are given in $\mu\text{g}/\text{kg}$.

Healing studies

The operation and treatments were well tolerated by all animals. Weight gain was similar in all six groups (*Table 2*). Neither treatment caused any damage to the gastric mucosa (data not shown).

Table 2. Animal weight in grams, mean \pm SEM of eight rats.

	DAY 1	DAY 7	DAY 15
STUDY I			
group 1	176.2 ± 5.0	201.2 ± 4.4	
group 2	173.7 ± 3.7	207.5 ± 3.1	
group 3	180.0 ± 4.9	206.2 ± 4.7	
STUDY II			
group 1	235.5 ± 3.4	236.9 ± 6.0	240.8 ± 6.5
group 2	230.1 ± 2.5	236.0 ± 4.4	251.9 ± 4.6
group 3	229.4 ± 3.2	245.5 ± 5.6	236.4 ± 5.5

Planimetrical ulcer area

STUDY I: Interleukin-1 did not modulate ulcer development (day 1) in either the non-antisecretory or the acid-inhibitory dose. Similarly, after one week ulcers in all three groups were decreased to approximately to 16% of their size 24 hours after induction (*Fig. 2*).

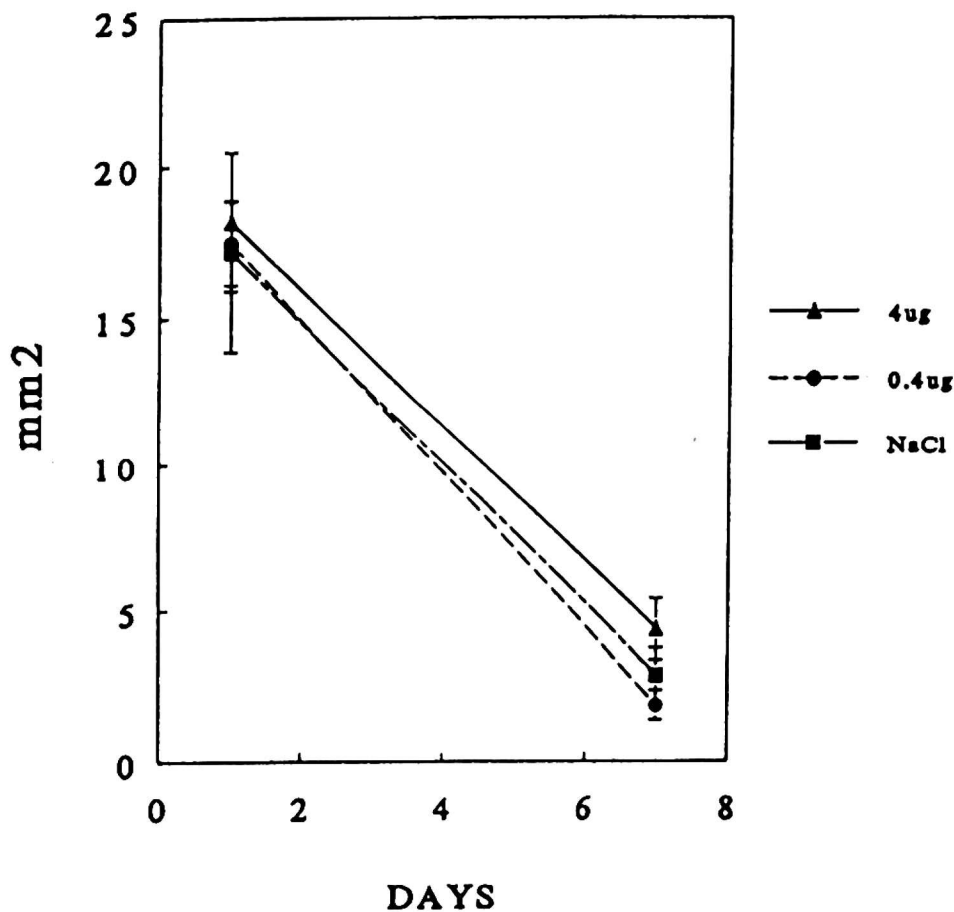


Fig. 2. STUDY I: Planimetrically determined ulcer size in μm , 1 and 7 days after ulcer induction. Doses of IL-1 are given in $\mu\text{g}/\text{kg}/\text{bid}$. Mean \pm SEM of eight animals.

STUDY II: The planimetrically determined ulcer size was similar in all groups one day after ulcer induction and showed a rapid and parallel decrease at the following checkpoints. All ulcers were almost completely healed at the end of the treatment period.

DISCUSSION

We hypothesized that since IL-1 exhibits mucosal protective as well as antisecretory capacities, IL-1 treatment could have resulted in smaller ulcers and accelerated healing while IL-1 inhibition with IX 207-887 could have resulted in an opposite effect. However, in this study IL-1 did not attenuate ulcer development or accelerate healing despite its established mucosal protective (6, 7) and its demonstrated antisecretory capacity (3, 9–11). Likewise

IL-1 inhibition with IX 207-887 did not impair ulcer development or delay ulcer healing.

The failure to attenuate ulcer development can be interpreted by the marked necrotizing tissue damage created by the cryoprobe, although this model has been demonstrated to be suitable for demonstration of protection, in connection with the evaluation of gastric mucosal protection by fish oil diet (16). Also the lack of effect on ulcer healing in this well established model is not fully unexpected since mucosal protection as demonstrated with prostaglandin treatment, does not necessarily translate into accelerated healing (5,17). Furthermore, in this study, IL-1 treatment, because of possible side effects, was given only at rather low doses and for a short time period. Since antisecretory mediated ulcer healing parallels the degree of acid inhibition (18, 19), it remains to be established whether higher doses or prolonged treatment will have an influence on peptic ulcer healing.

Since most substances used for therapy of rheumatic disease, especially non steroidal anti-inflammatory drugs (NSAID) which interfere with the endogenous prostaglandin synthesis, have side effects in the gastrointestinal tract, from mucosal damage up to the development of ulcerations (20), other strategies to counteract the process of inflammation have been sought. This need appears to be met by the development of IX 207-887, which in our investigations, even at a high dose, did not interfere with peptic ulcer healing or cause injury to the gastric mucosa and thus warrants further studies.

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