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Short Communication

Anti-ulcer activity of IAC, a novel free-radical scavenger, in rats

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Abstract

Objectives We investigated the ability of a novel low-molecular-weight free-radical scavenger, bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate (IAC), to protect the gastric mucosa from indometacin-induced ulceration.

Methods The pharmacological effects of IAC, administered orally or by intraperitoneal injection, on the gastric mucosa were assessed in a rat model of gastric ulceration induced by indometacin. The effect of IAC on the level of prostaglandin PGE_2 in the gastric mucosa was also investigated.

Results IAC, which has no ulcerative activity *per se*, had a preventive and protective activity against indometacin-induced gastric ulceration. This effect could be only partially attributed to a modulatory effect of IAC on PGE_2 levels; it is more likely to be due to the antioxidant activity of the compound.

Conclusions Taking into account the properties of IAC and the mechanisms underlying gastric inflammation elicited by non-steroidal anti-inflammatory drugs, IAC may represent a novel anti-ulcer agent.

Keywords IAC; indometacin; gastric ulcer; PGE_2; scavengers

Introduction

The stomach is vulnerable to damage by a variety of insults such as sepsis, trauma, multiple organ failure and non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs such as indometacin are widely used in the treatment of osteoarthritis and rheumatoid arthritis. However, their use is associated with a broad spectrum of side-effects in the gastrointestinal tract, such as bleeding and perforation.[1] Multiple pathogenetic elements underlie the development of these lesions, such as depletion of prostaglandins (PGs) through inhibition of cyclooxygenase (COX),[2–4] disturbances of the gastric microcirculation[5] and increased cell death by apoptosis.[6] Approaches to the prevention of gastric damage have included the suppression of acid secretion and the use of prostaglandin analogues, H_2-histamine receptor antagonists, proton pump (H^+/K^+ ATPase) inhibitors and, most recently, COX-2 inhibitors.[7]

NSAID gastropathy has been shown to result from neutrophil activation,[8] the formation of reactive oxygen species (ROS)[9] and the release of nitric oxide,[10] leading to new approaches to the prevention of NSAID-mediated gastric damage.

The new molecule bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate, called IAC (Figure 1), rapidly scavenges the majority of radical species involved in oxidative stress.[11] This compound has been shown to cross cell membranes and to distribute into biological environments without altering intracellular components. To date, IAC has been used in the quantitative measurement of ROS in biological samples from athletes[11] and patients with beta-thalassaemia.[12]

IAC has been shown to exert a protective effect *in vitro* in islets isolated from patients with type-2 diabetes, and *in vivo* in a non-obese diabetic mouse model.[13]

Given its antioxidant properties, the drug was tested for its potential anti-inflammatory activity. We have shown that IAC has a consistent effect in different animal models of inflammation (paper in preparation).

The main goal of the current work was to study the effect of IAC in the gastrointestinal tract, bearing in mind the well-known NSAID-induced gastropathy. We therefore tested the potential ulcerogenic activity of IAC following oral or intraperitoneal (i.p.) administration to rats. The results of the our earlier experiments prompted us to test the effect of IAC on indometacin-induced ulceration and in turn to evaluate the ability of the compound to

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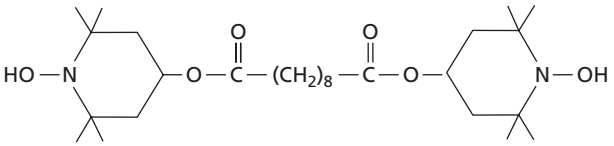

Figure 1 Structure of IAC, bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)-decandioate.

modulate levels of PGE_2 in the gastric mucosa. Here we report that IAC is devoid of gastrotoxicity *per se* and exerts a gastroprotective effect against indometacin-induced gastric lesions.

Materials and Methods

Animals

Male Sprague-Dawley rats (Harlan Italy, Udine, Italy) weighing 180–200 g were used for this study. The animals were housed at 22 ± 1°C and 65% humidity, with a 12 h light–dark cycle and water and food available *ad libitum*.

All procedures were performed in accordance with the guidelines of the National (D.L. n. 116/1992) and European legislation (EEC n. 86/609) and the National Institutes of Health on the use and care of laboratory animals.

Drugs

IAC was obtained from Medestea Research & Production (Turin, Italy). Indometacin was purchased from Sigma-Aldrich (Milan, Italy).

Experimental gastric ulcers

Rats were fasted for 24 h before starting the experiment and then randomly divided into groups of 6–10 animals. IAC alone was administered i.p. or orally at doses of 25 and 50 mg/kg; indometacin (15 mg/kg administered orally) was used as the reference NSAID to induce ulcers. IAC was also administered orally or i.p. at a dose of 50 mg/kg 15 min before indometacin.

Rats were sacrificed 6 h after treatment and the stomachs removed and opened along the greater curvature. The stomach contents were gently washed out with warm saline and the mucosa inspected for macroscopic gastric injury. The severity of lesions was scored as follows: 0 = normal mucosa; 1 = redness or a limited number of petechiae; 2 = mucosal erosions or a lot of petechiae or small ulcers (≤1 mm^2); 3 = up to three large ulcers (≥1 mm^2); 4 = more than three large ulcers; 5 = perforating ulcer.

Measurement of PGE_2 level in gastric mucosa

The gastric mucosa was dissected and homogenized in 0.1 M Na_2HPO_4, 1 mM EDTA ice-cold buffer containing 0.1 M indometacin, and centrifuged at 10 000 rpm at 4°C for 1 min. The supernatant was purified through a Sep-Pak C18 cartridge (Waters, Milan, Italy) pre-conditioned with ethanol and hexane, and eluted with ethylacetate. The eluent was evaporated under nitrogen gas and the residue dissolved in assay buffer. The concentration of PGE_2 was measured using an enzyme immunoassay kit, according to the manufacturer's instructions (Assay Design, Ann Harbor, USA).

Statistical analysis

Data are given as mean ± SEM. Statistical analysis was with the Kruskal–Wallis test and Dunn's multiple comparison test or one-way analysis of variance (ANOVA) followed by the Newman–Keuls post-hoc test. In all cases $P < 0.05$ was considered significant.

Results

As reported in Tables 1 and 2, indometacin induced gastric ulcers, with scores of 4.2 ± 0.1 and 3.8 ± 0.1 ($P < 0.01$ vs score with vehicle alone). IAC administered either i.p. or orally at doses of 25 and 50 mg/kg did not induce any alteration of the gastric mucosa. We then investigated the ability of IAC to prevent the ulcerogenic activity exerted by indometacin. As reported in Figure 2, indometacin had an ulcerogenic effect, leading to gastric ulcers with a score of 4.0 ± 0.1 ($P < 0.001$ vs vehicle score). IAC administered at 50 mg/kg i.p. 15 min before indometacin had a moderate protective effect against indometacin-induced gastric ulceration (2.5 ± 0.1 vs 4.0 ± 0.1). Oral treatment with IAC at the same dose completely prevented indometacin-induced gastric ulceration (0.8 ± 0.2 vs 4.0 ± 0.1, $P < 0.001$).

As shown in Figure 3, IAC did not affect PGE_2 levels when administered alone, whereas, as expected, indometacin markedly decreased the mucosal PGE_2 content of the gastric tissues, from 52.77 ± 3 ng/g to 6.49 ± 2 ng/g tissue after 6 h of exposure, in parallel with the presence of gastric ulceration. Treatment with IAC ameliorated the reduction due to indometacin, the concentration of PGE_2 being 16.49 ± 4 ng/g tissue.

Table 1 Effect of intraperitoneal IAC on gastric mucosal damage

Treatment	Gastric ulcer score
Vehicle	0.0 ± 0.0
Indometacin 15 mg/kg	4.2 ± 0.1**
IAC 25 mg/kg	0.4 ± 0.2
IAC 50 mg/kg	0.0 ± 0.0††

Values represent means ± SEM ($n = 6$).
**$P < 0.01$ vs vehicle; $^{\dagger\dagger}P < 0.01$ vs indometacin (Kruskal–Wallis test followed by Dunn's multiple comparison test).

Table 2 Effect of oral IAC on gastric mucosal damage

Treatment	Gastric ulcer score
Vehicle	0.0 ± 0.0
Indometacin 15 mg/kg	3.8 ± 0.1**
IAC 25 mg/kg	0.1 ± 0.1†
IAC 50 mg/kg	0.2 ± 0.2†

Values represent means ± SEM ($n = 6$).
**$P < 0.01$ vs vehicle; $^{\dagger}P < 0.05$ vs indometacin (Kruskal–Wallis test followed by Dunn's multiple comparison test).

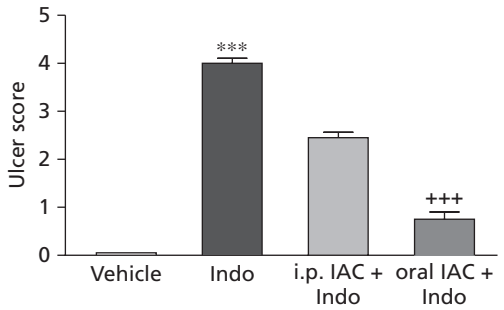

Figure 2 Effect of pretreatment with IAC (50 mg/kg) on indometacin (Indo)-induced gastric ulceration. Data represent means ± SEM (n = 10). ***$P < 0.001$ vs vehicle, $^{+++}P < 0.001$ vs indometacin-treated rats (Kruskal–Wallis test followed by Dunn's multiple comparison test).

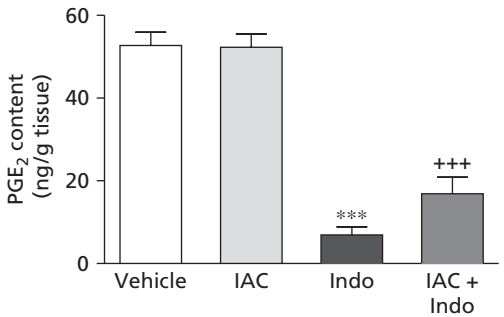

Figure 3 Effect of IAC (50 mg/kg i.p.) on prostaglandin PGE_2 levels in gastric mucosa after indometacin (Indo) treatment. Data represent means ± SEM (n = 6). ***$P < 0.001$ vs vehicle; $^{+++}P < 0.001$ vs indometacin-treated rats (one-way analysis of variance followed by the Newman–Keuls post-hoc test).

Discussion

It is well known that NSAIDs are often cytotoxic to gastric tissue. In previous experiments we found that IAC, a novel free-radical scavenger, had remarkable anti-inflammatory activity in different animal models of inflammation (paper in preparation). Given this property, we tested whether IAC protects against NSAID-induced gastric damage.

IAC administered i.p. before indometacin had a moderate protective effect against indometacin-induced gastric ulceration; oral pretreatment completely prevented indometacin-induced gastric ulceration. This difference might be due to a better bioavailability of the compound administered orally.

The effect elicited by IAC could mainly be attributed to its intrinsic antioxidant activity, which could explain its protective effect in indometacin-treated rats. Several studies have implicated the pro-oxidant activity of indometacin as the source of ROS in ulcerated tissues.[14] Moreover, the inhibition of PGE_2 synthesis by indometacin will lead to excessive production and accumulation of ROS.[15] IAC, which did not affect the level of PGE_2 in gastric mucosa *per se*, partially counteracted the decrease in PGE_2 elicited by indometacin. IAC did not restore the PGE_2 level altered by indometacin to normal, even though the PGE_2 level was significantly higher than in rats treated with indometacin alone. Thus, it seems reasonable to suggest that the protective effect exerted on gastric mucosa by this compound must be attributed to other mechanisms, such as the antioxidant activity of IAC.

Conclusions

These initial findings suggest that IAC has potential as a novel anti-ulcer medication. More experiments are necessary to evaluate other molecular determinants of IAC's activity.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

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References

1. Griffin MR *et al.* Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. *Ann Intern Med* 1991; 114: 257–263.
2. Whittle BJ. Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis, and the gastrointestinal damage induced by indometacin in the rat. *Gastroenterology* 1981; 80: 94–98.
3. Wallace JL *et al.* NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000; 119: 706–714.
4. Tanaka A *et al.* Role of cyclooxygenase (COX)-1 and COX-2 inhibition in nonsteroidal anti-inflammatory drug-induced intestinal damage in rats: relation to various pathogenic events. *J Pharmacol Exp Ther* 2002; 303: 1248–1254.
5. Suzuki K *et al.* Permissive role of neutrophils in pathogenesis of indometacin-induced gastric lesions in rats. *Med Sci Monit* 2000; 6: 908–914.
6. Slomiany BL *et al.* Induction of tumor necrosis factor-alpha and apoptosis in gastric mucosal injury by indometacin: effect of omeprazole and ebrotidine. *Scand J Gastroenterol* 1997; 32: 638–642.
7. Motawi TK *et al.* Gastroprotective effect of leptin in indometacin-induced gastric injury. *J Biomed Sci* 2008; 15: 405–412.
8. Wallace JL *et al.* Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990; 259: G462–G467.
9. Wallace JL. Pathogenesis of NSAID-induced gastroduodenal mucosal injury. *Best Pract Res Clin Gastroenterol* 2001; 15: 691–703.
10. Nishida K *et al.* Contribution of NO synthases to neutrophil infiltration in the gastric mucosal lesions in rats with water immersion restraint stress. *FEBS Lett* 1998; 425: 243–248.
11. Valgimigli L *et al.* Measurement of oxidative stress by EPR radical-probe technique. *Free Radic Biol Med* 2001; 31: 708–716.
12. Filosa F *et al.* Quantitative evaluation of oxidative stress status on peripheral blood in beta-thalassaemic patients by means of electron paramagnetic resonance spectroscopy. *Br J Pharmacol* 2005; 131: 135–140.
13. Novelli M *et al.* Reduction of oxidative stress by a new low-molecular-weight antioxidant improves metabolic alterations in a non-obese mouse diabetes model. *Pancreas* 2007; 35: 10–17.
14. Galati G *et al.* Idiosyncratic NSAID drug induced oxidative stress. *Chem Biol Interact* 2002; 142: 25–41.
15. Harada N *et al.* Activated neutrophils impair gastric cytoprotection role of neutrophil elastase. *Dig Dis Sci* 2000; 45: 1210–1216.