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Antiulcer activities of liquorice and its derivatives in experimental gastric lesion induced by ibuprofen in rats

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Abstract

Ibuprofen is a clinically important non-steroidal anti-inflammatory analgesic and antipyretic drug widely used in the treatment of several forms of arthritis and for mild to moderate pain. Analogously to other aspirin-like drugs, ibuprofen irritates the gastric mucosa and liquorice extracts have long been used to treat peptic ulcers. In this study we examined the protective effect of liquorice or its derivatives against gastric ulcers induced by oral ibuprofen. A granular mixture of ibuprofen alone or coated with liquorice or its derivatives including deglycyrrhized liquorice (DGL), highly glycyrrhized liquorice (HGL), enoxolone (glycyrrhetinic acid) and carbenoxolone, were studied. Ibuprofen coated with liquorice, DGL or enoxolone reduced the number and size of ulcers, lowering the ulcer index from 1.86 to 1 and the incidence from 100 to 59%. Coating with other derivatives was less effective. Plasma concentrations of ibuprofen were determined by high-performance liquid chromatography (HPLC), and showed that ibuprofen absorption was not affected by liquorice or its derivatives.

Keywords: Ibuprofen; Peptic ulcer; Liquorice; Deglycyrrhized liquorice; Enoxolone

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have antipyretic, analgesic and anti-inflammatory effects. In addition to sharing many therapeutic activities, NSAIDs exert several unwanted effects. The most common side effect is the causing of peptic ulcers that can sometimes be accompanied by anemia from the resultant blood loss (Insel, 1991). The physicochemical characteristics of these drugs (pK_a value, solubility, acidic pH of the environment) and biochemical parameters (inhibitory potency towards cyclooxygenase) or pharmacokinetic parameters (the degree of hepatic elimination of the unchanged drug and its phase II metabolites) contribute to the acute gastric and/or intestinal toxicity of these compounds (Beck et al., 1990).

Gastric damage by these agents is induced via at least two distinct mechanisms: local action by contact with the gastrointestinal mucosa and systemic action by inhibition of biosynthesis of gastric prostaglandins (PGs), especially PGI_2 and PGE_2.

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It has been shown that ibuprofen induces gastrointestinal toxicity by a local rather than a systemic mechanism (Cioli et al., 1979).

Liquorice root has long been used in medicine. It is used as a flavouring and sweetening agent, drug demulcent, expectorant and surfactant (Tylor et al., 1988). In addition, it has been reported that liquorice is effective in gastric ulcer treatment (Bennet et al., 1985; Tylor et al., 1988) and glycyrrhetinic acid, the aglycone of glycyrrhizin, has an anti-inflammatory and antiulcer effect (Yano et al., 1989). Liquorice and its derivatives possess cortisone-like action (Teelucksingh et al., 1991).

Deglycyrrhized liquorice protects against gastric ulcers in rats induced by pyloric ligation (Andersson et al., 1971; Marle et al., 1981) and against mucosal damage by bile in dogs and increased healing of peptic ulcers in patients (Rees et al., 1979).

Carbenoxolone sodium, the sodium salt of the 3β-*O*-hemisuccinate of glycyrrhetinic acid, has been extensively studied and reported to exert antiulcer effects in animals and man (Derelanko and Long, 1981; Parke, 1983; Peskar, 1983). Previously, we have shown that aspirin granules coated with liquorice or its derivatives reduce gastric mucosal damage in the rat (Dehpour et al., 1994).

In this investigation we have determined the effect of ibuprofen granules alone and coated with liquorice or its derivatives on ibuprofen induced gastric ulcers in rat.

2. Materials and methods

2.1. Materials

Ibuprofen, liquorice (containing more than 6.8% of glycyrrhizinic acid as measured by HPLC), deglycyrrhized liquorice (according to the BP standard, 1988), highly glycyrrhized liquorice (containing 15% glycyrrhizinic acid, prepared in our research laboratory), hydroxypropylmethylcellulose (HPMC) and sodium lauryl sulphate (SLS) were used. Mefenamic acid was employed as an internal standard, and acetonitrile (HPLC grade) and phosphate buffer (mobile phase) were used for the HPLC method.

Table 1
Granules prepared by the method of wet granulation, using HPMC and SLS

Formulations	1	2	3	4	5	6	7
Ibuprofen (g)	50	50	50	50	50	50	50
Liquorice (g)		25					
DGL (g)			25				25
HGL (g)				25			
Carbenoxolone (g)					1		1
Enoxolone (g)						1	

HPMC, hydroxypropylmethylcellulose; SLS, sodium lauryl sulphate. Liquorice containing more than 6.8% glycyrrhizinic acid was measured by HPLC. Deglycyrrhized liquorice (DGL): according to BP standard (1988). Highly glycyrrhized liquorice (HGL): the same derivatives as liquorice, but containing 15% glycyrrhizinic acid, prepared in the Research Laboratory.

2.2. Preparation of granules

Ibuprofen alone or coated with liquorice or its derivatives including DGL, HGL, carbenoxolone and their mixture were prepared by a wet granulation method (Rundic, 1990). In brief, the granules were prepared by mixing ibuprofen powder with liquorice powder or its derivatives and sodium lauryl sulphate (SLS) in a ribbon mixer for 20 min, then HPMC, granulating liquid, in distilled water was slowly added to the mixture and mixed until a wet mass was obtained. The wet mass was forced through a 14 mesh screen and the granules were dried by hot air circulation (60°C) except for carbenoxolone (heat-sensitive) which was dried by exposure to the air and then the granules were passed through a 25 mesh screen (Table 1).

2.3. Oral administration to animals

Male and female albino Wistar rats weighing 150–250 g were fasted for 24 h, but allowed free access to water. The granules were suspended in normal saline (30 mg ibuprofen per ml) and administered orally with a feeding needle as a single dose of 60 mg/kg. A control group received an equal volume of saline. After 4 h the rats were

killed with ether and the stomachs were excised immediately, cut along the greater curvature and the mucosa washed under a constant stream of water. Mucosal damage was assessed macroscopically without knowledge of the treatment administered.

In the other groups, the rats were administered orally the same amount of ibuprofen granules alone or coated with liquorice or some derivatives as a single dose, respectively. They were killed after 1 h and blood samples were collected for determination of ibuprofen plasma

Table 2
Degree of gastric mucosal damage produced by ibuprofen alone or coated with liquorice or its derivatives

Group	Rats (no.)	Mucosa lesions (gross findings)			Ulcer index (mean ± SE)	Percent incidence
Ibuprofen	29	Grade	0	3 (10.3%)	1.86 ± 0.02	90
			1	8 (27.5%)		
			2	11 (37.9%)		
			3	4 (13.7%)		
			4	3 (10.3%)		
Ibuprofen + liquorice [a]	28		0	11 (39.3%)	1.17 ± 0.22	61
			1	6 (21.4%)		
			2	7 (25.0%)		
			3	3 (10.7%)		
			4	1 (3.5%)		
Ibuprofen + DGL [b,d]	23		0	10 (43.4%)	1.04 ± 0.25	56
			1	6 (26.1%)		
			2	5 (21.7%)		
			3	0 (0.0%)		
			4	2 (8.7%)		
Ibuprofen + DGL + carbenoxolone [c]	17		0	0 (0.0%)	1.82 ± 0.21	100
			1	7 (41.1%)		
			2	7 (41.1%)		
			3	2 (11.7%)		
			4	1 (5.9%)		
Ibuprofen + enoxolone [a]	17		0	7 (41.1%)	1.0 ± 0.24	59
			1	4 (23.5%)		
			2	5 (29.4%)		
			3	1 (5.9%)		
			4	0 (0.0%)		
Ibuprofen + carbenoxolone [c]	18		0	4 (22.2%)	1.77 ± 0.32	77
			1	4 (22.2%)		
			2	4 (22.2%)		
			3	4 (22.2%)		
			4	2 (11.1%)		
Ibuprofen + HGL [c,e]	24		0	6 (25.0%)	1.79 ± 0.29	75
			1	5 (20.8%)		
			2	4 (16.7%)		
			3	6 (25.0%)		
			4	3 (12.5%)		

Severity of gastric mucosal damage was graded as follows: grade 0, no lesion; grade 1, less than five punctiform lesions (less than 1 mm); grade 2, punctiform lesions more than five or one small ulcer (1–2 mm); grade 3, many small ulcers or single linear ulcer of marked size. The ulcer index for each group was calculated by multiplying the number of rats in each grade by the number and dividing by the total number of rats in each group (Cioli et al., 1979; Azuumi et al., 1980).
[a] $p < 0.05$, [b] $p < 0.02$, [c] not significant, [d] glycyrrhized liquorice, [e] highly glycyrrhized liquorice.

level by HPLC. Plasma samples were stored at −20°C for a few days prior to HPLC assay for drug concentrations.

2.4. Assay of ibuprofen in plasma

The HPLC method employed was that reported by Satterwite and Boudinot (1989), as modified slightly. Standard solutions of ibuprofen were 200, 100 and 10 μg/ml in 0.01 M phosphate buffer at pH 6.0, containing 2.0, 1.0 and 0.1% of acetonitrile, respectively. Internal standard solutions of mefenamic acid were 10 μg/ml in 0.01 M phosphate buffer at pH 6.0, containing 0.1% of 1.0 M sodium hydroxide. Standard solution was added to rat plasma to provide concentrations of 5–100 μg/ml ibuprofen. Ibuprofen was extracted with this procedure: samples of plasma (100 μg) were placed in glass centrifuge tubes followed by the addition of 0.05 ml of 4.0 M perchloric acid. The tubes were vortex-mixed, followed by the addition of 0.5 M phosphate buffer at pH 2.0 to obtain a final volume of 2 ml. The tubes were again vortex-mixed, followed by the addition of 5 ml of a isooctane-isopropanol mixture (90:10, v/v). The mixture was shaken for 15 min and centrifuged at 2000 rpm for 10 min. The upper organic layer was removed and evaporated under a stream of nitrogen gas.

The residue reconstituted with 300 μg of mobile phase (30% of acetonitrile + 70% of 0.04 M phosphate buffer at pH 7.0) and 50 μl of the solution was then injected into the HPLC system. The HPLC conditions were as follows: pump and detector, Perkin Elmer series 410-Lc pump equipped with a Perkin Elmer Lc 950 UV/visible detector; column, a 25 cm × 0.46 cm stainless-steel column, C18-sil-X-5; integrator, Perkin Elmer LCI-100; flow rate, 1.1 ml/min; sensitivity, 0.05 a.u.f.s; detection, at 223 nm.

3. Results and discussion

The severity of mucosal damage produced by ibuprofen alone or coated with liquorice or its derivatives, as measured by the number and size of ulcer is listed in Table 2.

Liquorice, DGL and enoxolone reduced the size and number of ulcers in gastric mucosa significantly whereas other derivatives were not effective. Liquorice and its derivatives have been used to treat peptic ulcers; they are thought to act on the mucosa (Feldman and Gilat, 1971; Rees et al., 1979). DGL reduced bile acid-induced hydrogen ion back diffusion across canine gastric mucosa and diminished acute gastric mucosal damage due to aspirin alone or in combination with taurodeoxycholic acid (Morgan et al., 1983). Previous exposure of the gastric mucosa to deglycyrrhized liquorice did not significantly affect the degree of aspirin-induced damage. This suggests that its protective effect may be temporary, being diminished as a result of leaving the stomach or delayed because of absorption and distribution (Morgan et al., 1983). Other derivatives of liquorice such as carbenoxolone and glycyrrhetinic acid, have a protective effect on gastric mucosa (Downer et al., 1970; Bianchi et al., 1989). Carbenoxolone, like prostaglandins (PGs), is cytoprotective to the rat gastric mucosa. The mechanism of action is unknown (Derelanko and Long, 1981).

It has been reported that prostanoids are un-

Table 3
Plasma concentration of ibuprofen (μg/ml) as measured by HPLC (Satterwite and Boudinot, 1989) in animals given ibuprofen granules alone or coated with liquorice or its derivatives (each value represents the mean ± SE; $n = 4$)

Ibuprofen	Ibuprofen + liquorice	Ibuprofen + DGL	Ibuprofen + enoxolone
30.48 ± 0.15	42.8 ± 2.5 [a]	35.38 ± 6.4 [c]	39.03 ± 0.75 [b]

[a] $p < 0.01$.
[b] $p < 0.001$.
[c] Not significant.

likely to play an important role in the antiulcer activity of carbenoxolone and DGL in the rat. Even though prostaglandins may be involved in the action of carbenoxolone in man, various other mechanisms may also participate such as alteration of cell turnover and stimulation of mucus secretion. Similar actions also occur in the rat (Bennet et al., 1985). On the other hand, it has been shown that carbenoxolone accelerates the healing of gastric ulcers and prevents erosions caused by stress but not those caused by aspirin (Van Huis and Kramer, 1981) and carbenoxolone has been reported to be ineffective when given at the same time as or 1 min before the ethanol in ethanol-induced lesions in the rat (Derelanko and Long, 1981). In our study carbenoxolone dose not prevent gastric mucosal damage caused by ibuprofen.

We have previously shown that aspirin coated with liquorice or its derivatives reduced the number and size of ulcers (Dehpour et al., 1994).

As is evident from Table 3, coating the ibuprofen granules by liquorice, enoxolone and DGL not only does not prevent the oral absorption of ibuprofen but also increases its absorption. Therefore, it can be concluded that prevention of ulcer formation by these derivatives cannot be attributed to the decreased absorption of oral ibuprofen.

Our results show that ibuprofen coated with liquorice or some of its derivatives produced less gastric mucosal injury compared with ibuprofen alone.

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