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The protective effect of liquorice components and their derivatives against gastric ulcer induced by aspirin in rats

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Abstract—We have examined the protective effect of liquorice or its derivatives against gastric ulcer induced by aspirin. A granular mixture of aspirin alone and coated with liquorice or its derivatives including the deglycyrrhized form, a high glycyrrhized form, carbenoxolone, and enoxolone were studied. Aspirin coated with liquorice reduced the number and size of ulcers, reducing the ulcer index from $1{\cdot}5 \pm 0{\cdot}12$ to $0{\cdot}5 \pm 0{\cdot}12$ and the incidence from 96% to 46%. Coating with derivatives was less effective (ulcer index, 0·70–0·94; incidence 62–76%).

Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, possess excellent analgesic, antipyretic and anti-inflammatory effects and are widely accepted in daily practice. However, these drugs also induce erosion and ulcers on gastrointestinal mucosa (Flower et al 1985; Fujii et al 1988). The common characteristic of these drugs is their ability to inhibit cyclo-oxygenase, which results in the inhibition of prostaglandin synthesis (Robert et al 1979). As a mucosal protective factor, prostaglandin plays an important role in the prevention of ulceration (Fujii et al 1988).

Liquorice, the root and subterranean stem of different varieties of *Glycyrrhiza glabra*, has long been used in medicine. Besides being a valuable flavouring and sweetening agent, the drug has demulcent, expectorant, and antispasmodic action. In addition it has been shown that liquorice is effective in gastric ulcer treatment and has a cortisone-like action in rheumatic arthritis and other inflammatory diseases. Simple derivatives of β-glycyrrhetinic acid such as the disodium salt of carbenoxolone have been used extensively in gastric ulcer treatment (Habib et al 1979). Deglycyrrhized liquorice also lessens gastric mucosal damage (Bennett et al 1980).

In this study we have determined the effect of aspirin alone and coated with liquorice or its derivatives on aspirin-induced gastric ulcers in rats.

Materials and methods

Male albino Wistar rats, 200–250 g, were fasted for 24 h but permitted free access to water. Aspirin alone or coated with liquorice or its derivatives including the deglycyrrhized form, a high glycyrrhized form, carbenoxolone, enoxolone and their mixtures were prepared by a wet granulation method (Rudnic 1990); in brief, the granules were prepared by mixing aspirin powder with liquorice powder or its derivatives and sodium lauryl sulphate in a ribbon mixer for 25 min. The granulating liquid consisting of hypromellose (hydroxypropylmethylcellulose) and polyvinylpyrrolidone in 96% ethanol was slowly added to the mixture and mixed for 10 min. The wet mass was forced through a 16 mesh screen and the granules were dried by hot air circulation (60°C) and passed through a 25 mesh screen (Table 1).

The granules were suspended in 0·9% NaCl (saline) (equivalent to 26·6 mg mL^{-1} aspirin) and administered orally by gavage as a single dose of 266 mg kg^{-1}. A control group received an equivalent volume of saline. The animals were killed by exposure to ether, 4 h after dosing; the stomachs were excised immediately and cut along the greater curvature. The mucosal damage was assessed macroscopically.

In the experimental group, the rats were administered orally the same amount of aspirin granules alone or coated with liquorice or its derivatives as a single dose. They were killed after 20 or 60 min and blood samples were collected for determination of aspirin in serum by the method of Trinder (1954); this is a rapid method for the determination of salicylate in biological fluids based on a reagent containing ferric nitrate, mercuric chloride and hydrochloric acid, which precipitates the proteins and simultaneously reacts with salicylic acid to give a purple colour measured spectrophotometrically at 540 nm.

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Results

A quantitative assessment of gastric damage produced by aspirin alone or coated with liquorice or its derivatives, as measured by the number and size of ulcer, is shown in Table 2.

Liquorice or its derivatives significantly reduced the size and number of ulcers in gastric mucosal.

Aspirin absorption was not affected by liquorice or its derivatives (Table 3).

Discussion

Liquorice and its derivatives have long been used to treat peptic ulceration. Carbenoxolone speeds the healing of human gastric ulcers and protects against aspirin-induced depression of gastric mucosal potential difference. Deglycyrrhized liquorice protects against gastric ulceration in rats induced by pyloric ligation and some studies report increased healing of peptic ulcers in patients (Feldman & Gilat 1971; Rees et al 1979). Deglycyrrhinized liquorice 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, but did not greatly affect the aspirin absorption (Morgan et al 1983). Other derivatives of liquorice such as carbenoxolone and enoxolone (glycyrrhetinic acid), have a protective effect on gastric mucosa (Downer et al 1970; Bianchi et al 1989). Carbenoxolone sodium is considered to act by increasing the life-span of gastric epithelial cells, increasing

Table 1. Composition of formulated granules per 50 g aspirin.

	Liquorice[a]	DGL[b]	HGL[c]	Carbenoxolone	Enoxolone
1					
2	25				
3	25			1	
4		25			
5		25		1	
6			25		
7				1	
8					1

[a]Liquorice containing more than 6·8% glycyrrhizinic acid measured by HPLC. [b]Deglycyrrhized liquorice: according to BP standard (1988). [c]High glycyrrhized liquorice: the same derivatives as liquorice, but with added 15% glycyrrhizinic acid.

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

Formulation (number of rats)	Grade	Mucosal lesions (gross findings)	Ulcer index	Incidence (%)
1	0	2 (4)[a]	1·5	96
(46)	1	24 (52)	±0·12	
	2	15 (33)		
	3	3 (7)		
	4	2 (4)		
2	0	13 (54)	0·5***	46
(24)	1	10 (42)	±0·12	
	2	1 (24)		
	3	0 (0)		
	4	0 (0)		
3	0	4 (23)	0·94**	76
(17)	1	10 (59)	±0·15	
	2	3 (17)		
	3	0 (0)		
	4	0 (0)		
4	0	12 (35)	0·7***	65
(34)	1	20 (59)	±0·09	
	2	2 (6)		
	3	0 (0)		
	4	0 (0)		
5	0	8 (28)	0·82***	71
(28)	1	17 (61)	±0·11	
	2	3 (11)		
	3	0 (0)		
	4	0 (0)		
6	0	9 (37)	0·7***	62
(24)	1	13 (54)	±0·12	
	2	2 (8)		
	3	0 (0)		
	4	0 (0)		
7	0	8 (36)	0·81*	64
(22)	1	11 (50)	±0·16	
	2	2 (9)		
	3	1 (4)		
	4	0 (0)		
8	0	8 (30)	0·74***	70
(27)	1	18 (67)	±0·10	
	2	1 (4)		
	3	0 (0)		
	4	0 (0)		

Severity of gastric mucosal damage was graded as follows: 0–no lesion; 1–haemorrhagic erosion (less than 5); 2–haemorrhagic erosion (more than 5) or one small ulcer; 3–many small linear ulcers (shorter than 2 mm) or single linear; ulcer of marked size (longer than 2 mm); 4–multiple linear ulcers 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 (Azuumi et al 1980). [a]Numbers in parentheses are percentages. *$P<0.01$, **$P<0.02$, ***$P<0.001$ compared with uncoated aspirin.

mucus production, decreasing hydrogen ion back-diffusion, inhibiting peptic activity, and stimulating immunoreactive secretin production (Nagy 1978).

Moreover, coating the aspirin granules by liquorice or its derivatives not only does not prevent the absorption of oral aspirin, but may even increase its absorption (Table 3). Therefore, prevention of ulcer formation by these derivatives cannot be attributed to the decreased absorption of oral aspirin.

Our results show that aspirin coated with liquorice or its derivatives, produced less gastric mucosal injury compared with aspirin alone.

Table 3. Serum salicylic acid level as measured by the method of Trinder (1954) in animals given aspirin granules alone or coated with liquorice or its derivatives.

	Serum salicylic acid	
Formulation	20 min	60 min
1	31·30±2·88	30·14±1·30
2	34·42±2·30	28·49±1·15
3	34·71±3·09	41·42**±1·32
4	35·09±1·15	33·60±1·73
5	36·69±1·49	35·20±2·27
6	32·53±0·74	35·62*±0·74
7	38·20±1·73	35·73±2·30
8	30·56±2·12	29·60±2·02

*$P<0.05$, **$P<0.01$ compared with uncoated aspirin (Formulation 1). Each test used at least five rats. Each value represents the mean±s.e.m.

References

Azuumi, Y., Ohara, S., Ishihara, K., Okabe, H., Hotta, K. (1980) Correlation of quantitative changes of gastric mucosal glycoproteins with aspirin-induced gastric damage in rats. Gut 21: 533–536

Bennett, A., Clark-Wibberley, T., Stamford, I. F., Wright, J. E. (1980) Aspirin-induced gastric mucosal damage in rats: cimetidine and deglycyrrhizinated liquorice together give greater protection than low doses of either drug alone. J. Pharm. Pharmacol. 32: 151

Bianchi, P., Tubaro, A., Negro, P. Del., Dellaloggia, R. (1989) Improvement of the topical anti-inflammatory activity of glycyrrhetinic acid by complexation with phospholipids: an histological approach. Pharmacol. Res. 21: 483–484

British Pharmacopoeia (1988), pp 674–675.

Downer, H. D., Galloway, R. W., Horwich, L., Parke, D. V. (1970) The absorption and excretion of carbenoxolone in man. J. Pharm. Pharmacol. 22: 479–487

Feldman, H., Gilat, T. (1971) A trial of deglycyrrhizinated liquorice in the treatment of duodenal ulcer. Gut 12: 449–451

Flower, R. J., Moncada, S., Vane, J. R. (1985) Drug therapy of inflammation, analgesic-antipyretics and anti-inflammatory agents; drugs employed in the treatment of gout. In: Gilman, A. G., Goodman, L. S., Rall, T. W., Murad, F. (eds) The Pharmacological Basis of Therapeutics. Macmillan Publishing Company, New York, pp 674–715

Fujii, A., Kuboyama, N., Kobayashi, S., Namiki, Y., Tamura, T. (1988) Time-course study of gastric damages in rats by anti-inflammatory drugs using a gastroscope and its quantification. Jpn. J. Pharmacol. 48: 317–322

Habib, A. A. M., El-Sebakhy, N. A., Kadry, H. A. (1979) New and simple methylene blue colorimetric assay for glycyrrhizin in pharmaceuticals. J. Pharm. Sci. 68: 1221–1223

Morgan, R. J., Nelson, L. M., Russell, R. I., Docherty, C. (1983) The protective effect of deglycyrrhinized liquorice against aspirin and aspirin plus bile acid-induced gastric mucosal damage, and its influence on aspirin absorption in rats. J. Pharm. Pharmacol. 35: 605–607

Nagy, G. S. (1978) Evaluation of carbenoxolone sodium in the treatment of duodenal ulcer. Gastroentrology 74: 7–10

Rees, W. D. W., Rhodes, J., Wright, J. E., Stamford, I. F., Bennett, A. (1979) Effect of deglycyrrhizinated liquorice on gastric mucosal damage by aspirin. Scand. J. Gastroent. 14: 605–607

Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl, and thermal injury. Gastroenterology 77: 433–443

Rudnic, E. (1990) Oral solid dosage forms. In: Gennaro, A. R., Chase, G. D., Marderosian, A. D., Harvey, S. C., Hussar, D. A., Medwick, T., Rippie, E. G., Schwartz, J. B., Swinyard, E. A., Zink, G. L. (eds) Remington's Pharmaceutical Sciences. 18th edn, Mack Publishing Company, pp 1641–1644

Trinder, P. (1954) Rapid determination of salicylate in biological fluids. Biochem. J. 57: 301–303