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Paracetamol Confers Resistance to Ethanol-induced Gastric Mucosal Damage in Rats

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Abstract—Paracetamol given orally or subcutaneously did not produce any observable gastric mucosal damage, nor did it change the acidity of the residual secretion in rat stomachs. However, it delayed the gastric emptying rate and increased the residual volume of gastric secretion. Pretreatment with paracetamol 250 mg kg^{-1} significantly prevented ethanol-induced gastric ulceration. Although it did not influence ethanol-stimulated acid secretion, it increased the mucosal mucus content in the ethanol-treated animals. The findings suggest that the protective mechanism of paracetamol against ethanol-induced damage is likely to be due to improved gastric mucosal integrity, through an increase in the adherent mucosal mucus which protects the underlying delicate cellular structures.

Paracetamol is analgesic and antipyretic but differs from many non-steroidal anti-inflammatory drugs (NSAIDs) in having no significant anti-inflammatory activity. In its selective inhibitory action on prostaglandin synthesis in the brain (Tolman et al 1983), it is also different from NSAIDs which have a profound gastrointestinal-damaging effect in man and in animals (Pfeiffer & Lewandowski 1971; Bartle et al 1986; Hawkey et al 1986). Oral administration of a high dose of paracetamol to rats has been found to antagonize stomach mucosal ulceration by various gastric irritants, such as ethanol, sodium hydroxide, hydrochloric acid and NSAIDs (Seegers et al 1979; Van Kolfschoten et al 1983); this has not been seen in dogs (Leeling et al 1981) or in man (Lanza et al 1986). Increased prostaglandin synthesis in gastric tissue has been thought to be responsible for the antiulcer effect of paracetamol (Van Kolfschoten et al 1981, 1983). However, it is still unclear whether the drug is involved in peripheral tissue prostaglandin synthesis because of the negative findings of McDonald-Gibson & Collier (1979).

The object of the present study has been to examine whether doses of paracetamol less than those used by Van Kolfschoten et al (1983) have an antiulcer action when given orally (p.o.) or subcutaneously (s.c.) to rats with ethanol-induced gastric damage. Also investigated were the pharmacological actions of the drug on gastric mucus content, emptying rate, residual volume and acid level in the absence or presence of ethanol.

Methods

General

Male Sprague-Dawley rats (200±20 g) were reared on a standard laboratory pellet diet (Ralston Purina Co.) and drank tap water. They were starved for 24 h before experimentation but were allowed free access to tap water; this was removed 1 h before administration of paracetamol (Sigma). All experiments were conducted in an air-conditioned room (temperature 22±1 °C, relative humidity 65–70%) where the animals were normally housed.

Following 24 h starvation, the rats were given paracetamol suspended in 4% Tween 80 (Sigma) either p.o. via a stainless steel gastric tube or s.c., in a volume of 5 mL kg^{-1}. This was followed 30 min later by 40% v/v ethanol (Merck) in distilled water, 10 mL kg^{-1} p.o. Rats acting as controls were given the appropriate vehicle in a similar volume and by the same route. All animals were killed by a sharp blow on the head 1 h after ethanol treatment.

Measurement of the gastric ulcer index

Stomachs, removed immediately after killing the rats, were opened along the greater curvature and ulcer size measured using a grid (each grid was 1 mm^2) placed on the glandular mucosal surface (Ogle et al 1985); measurements were carried out by an observer who was unaware of the treatment regimen. In the case of petechiae, five such lesions were recorded as the equivalent of 1 mm^2. The sum of the ulcer areas in each group of rats was divided by the number of animals and expressed as the mean ulcer index.

Determination of the gastric wall mucus content

The amount of mucus adhering to the glandular mucosa of other groups of rats was measured by the Alcian blue method (Corne et al 1974). Following removal and opening the stomachs along their greater curvatures, each organ was immediately transferred to 10 mL of 0·1% w/v buffered Alcian blue (Sigma) solution (pH 5·8) where it was left for 2 h at room temperature (20 °C). Excess (uncomplexed) dye was removed by two successive washes, each in 0·25 M sucrose (BDH), lasting 15 and 45 min. The glandular portion of the stomach was then carefully cut away from the whole tissue. Dye complexed with the glandular mucosal wall mucus was eluted by immersion in 10 mL aliquots of 0·5 M $MgCl_2$ (Merck) which were intermittently shaken for 10 min at 1 h intervals for 2 h in a flask shaker (Stuart Scientific Co. Ltd). 5 mL of the blue solution were then shaken briefly with an equal volume of diethyl ether (BDH) and the optical density of the aqueous phase measured at 598 nm with a spectrophotometer (Beckman DB-G). The quantity of Alcian blue extracted per g wet glandular tissue was calculated from

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standard curves which obeyed the Beer-Lambert Law at the dye concentrations used.

Determination of the gastric emptying rate and residual secretory acid and volume

Experiments were done on separate groups of rats. Paracetamol was given 30 min before oral administration of distilled water or 40% ethanol via a stainless steel gastric tube. Twenty pellets of Amberlite 1R-120 (Fisher Scientific Co.), approximately 0·5 mm in diameter, were given together with the distilled water or 40% ethanol (the resin did not remove gastric acid or small amounts of paracetamol).

Stomachs were removed 1 h later, after clamping both the oesophageal and pyloric ends. Each organ was opened, along its greater curvature, over a glass funnel for the gastric juice to be collected in a graduated centrifuge tube and the volume recorded. The mucosal surface was then rinsed with distilled water, which was added to the collected gastric juice. The acid content of the gastric juice was measured by titration with 0·01 M NaOH, using an autotitration system (Radiometer model TTT 80). The number of pellets remaining in the stomach was counted. All data were analysed by Student's two-tailed *t*-test.

Results

Tables 1 and 2 show the results of acute paracetamol pretreatment given p.o. and s.c., respectively, on ethanol-induced glandular mucosal damage and changes in gastric wall mucus content. Administration of a single oral dose of ethanol produced severe linear haemorrhagic gastric ulcers and a small number of petechiae. Paracetamol pretreatment with 250 mg kg^{-1}, given either p.o. or s.c., significantly reduced ethanol-induced mucosal ulceration. The mucus level in the glandular mucosa was not affected by ethanol or paracetamol treatment alone. However, simultaneous administration of paracetamol and ethanol significantly increased the gastric mucus level.

Tables 3 and 4 display the results of acute paracetamol pretreatment given p.o. and s.c., respectively, on gastric emptying rate as well as the volume and acid level of residual gastric secretion. Gastric emptying rate was significantly reduced, especially in the p.o.-pretreated groups. Pretreatment s.c. with the drug had a relatively less marked effect, only the high dose (250 mg kg^{-1}) was found to lower the emptying rate significantly. Furthermore, the action of paracetamol on gastric emptying was not delayed further by oral administration of ethanol, although the latter itself markedly lowered the emptying rate.

Oral administration of paracetamol 125 or 250 mg kg^{-1} significantly increased the residual volume of gastric secretion; however, only the big s.c. dose of paracetamol increased this parameter (Tables 3, 4). Ethanol also markedly elevated the volume of residual gastric content and this was enhanced by paracetamol pretreatment p.o. or s.c., in a dose-dependent manner (Tables 3, 4). Paracetamol did not influence the basal acid content in the residual gastric secretion, irrespective of its route of administration, nor did it affect the stimulatory action of ethanol on acid output (Tables 3, 4).

Table 1. Effects of acute paracetamol pretreatment (given p.o. 30 min before oral administration of ethanol) on ethanol-induced gastric glandular ulceration and mucus level changes (rats killed 1 h after ethanol).

Pretreatment (p.o.)	Ulcer index (mm^2)	Mucus content (μg Alcian blue g^{-1} wet wt tissue)
A. Distilled water 10 mL kg^{-1} p.o.		
Tween 80 4% 5 mL kg^{-1}	0·28 ± 0·09 (12)	423·26 ± 22·87 (10)
Paracetamol 125 mg kg^{-1}	0·30 ± 0·08 (12)	374·85 ± 19·73 (10)
Paracetamol 250 mg kg^{-1}	0·44 ± 0·10 (12)	376·49 ± 17·92 (10)
B. Ethanol 40% 10 mL kg^{-1} p.o.		
Tween 80 4% 5 mL kg^{-1}	12·65 ± 2·55† (13)	429·92 ± 18·50 (10)
Paracetamol 125 mg kg^{-1}	12·10 ± 2·93† (16)	411·39 ± 15·86 (10)
Paracetamol 250 mg kg^{-1}	0·94 ± 0·35* (12)	448·87 ± 13·21† (10)

Values indicate means ± s.e.m.
Figures in parentheses indicate the number of rats used in each group.
*$P < 0·01$ when compared with its own control pretreated p.o. with 4% Tween 80.
†$P < 0·01$ when compared with the corresponding group given distilled water p.o. in A.

Discussion

Paracetamol given either p.o. or s.c. antagonized ethanol-induced gastric damage. These findings not only support but also extend those of Van Kolfschoten et al (1983) who used doses of 500 mg kg^{-1} given p.o. Paracetamol was found in the present study to have an antiulcer action when given s.c.; furthermore, this antiulcer effect occurred when a dose of 250 mg kg^{-1} was used. Since s.c. administration with paracetamol protected against the gastric damaging effect of ethanol, this finding tends to argue against the possibility of a topical antiulcer action of the drug being due to the known mechanism where a mild irritant can exert a generalized protective action against the subsequent damaging effect of a more concentrated noxious irritant (Danon & Assouline 1979; Robert 1980). The ability of the drug to act on the stomach following its s.c. absorption into the systemic circulation indeed supports the idea that the antiulcer action of paracetamol is specific and is not due to a mild irritant action through direct contact of the drug with the stomach mucosa.

It is known that gastric acid plays a role in stomach ulceration (Alphin et al 1977). However, the antiulcer action of paracetamol appears not to be mediated by reduced acid secretion (Tables 3, 4). Also, it is unlikely that protection by paracetamol is due to accelerated gastric emptying of ethanol which shortens the exposure period of the gastric mucosa to the damaging agent, because paracetamol itself decreased the stomach emptying rate to a similar degree as did ethanol (Tables 3, 4). Furthermore, simultaneous administration of both drugs did not slow further the emptying rate of the resin pellets. These findings suggest that paracetamol and ethanol may act either to decrease stomach contraction (Koo et al 1985, 1986) or cause pyloric spasm (Goldstein 1983), both of which can decrease gastric emptying rate.

A high concentration of ethanol has been shown to deplete gastric mucus (Bell et al 1985). The low dose of ethanol, used in the present study, did not have a mucus-depleting effect, and confirms the finding of Wong et al (1986). Although paracetamol itself did not influence the mucus level, it elevated this parameter in ethanol-treated rats. Increased

Table 2. Effects of acute paracetamol pretreatment (given s.c. 30 min before oral administration of ethanol) on ethanol-induced gastric glandular ulceration and mucus level changes (rats killed 1 h after ethanol).

Pretreatment (s.c.)	Ulcer index (mm^2)	Mucus content (μg Alcian blue g^{-1} wet wt tissue)
A. Distilled water 10 mL kg^{-1} p.o.		
Tween 80 4% 5 mL kg^{-1}	0·16 ± 0·05 (16)	431·13 ± 18·43 (10)
Paracetamol 125 mg kg^{-1}	0·10 ± 0·05 (12)	444·55 ± 26·72 (10)
Paracetamol 250 mg kg^{-1}	0·12 ± 0·10 (12)	391·05 ± 30·50 (10)
B. Ethanol 40% 10 mL kg^{-1} p.o.		
Tween 80 4% 5 mL kg^{-1}	15·01 ± 2·08 (23)	451·39 ± 18·44 (10)
Paracetamol 125 mg kg^{-1}	11·55 ± 2·75^{+} (19)	490·77 ± 30·02 (10)
Paracetamol 250 mg kg^{-1}	3·22 ± 0·75^{*+} (17)	568·30 ± 26·49^{*+} (10)

Values indicate means ± s.e.m.
Figures in parentheses indicate the number of rats used in each group.
$^{*}P < 0·01$ when compared with its own control pretreated s.c. with 4% Tween 80.
$^{+}P < 0·01$ when compared with the corresponding group given distilled water p.o. in A.

Table 3. Effects of acute paracetemol pretreatment (given p.o. 30 min before oral administration of ethanol) on ethanol-induced gastric emptying rate and gastric volume and acid level changes (rats killed 1 h after ethanol).

Pretreatment (p.o.)	Emptying rate (% of pellets expelled)	Gastric volume (mL/100 g h^{-1})	Acid content (μequiv/100 g h^{-1})
A. Distilled water 10 mL kg^{-1} p.o.			
Tween 80 4% mL kg^{-1}	62·50 ± 7·80 (12)	0·00 ± 0·00 (12)	9·94 ± 1·15 ± 1·15 (10)
Paracetamol 125 mg kg^{-1}	12·10 ± 5·10* (12)	0·29 ± 0·09* (12)	11·60 ± 1·70 (12)
Paracetamol 250 mg kg^{-1}	15·80 ± 6·50* (12)	0·80 ± 0·06* (12)	13·08 ± 2·00 (12)
B. 40% ethanol 10 mL kg^{-1} p.o.			
Tween 80 4% 5 mL kg^{-1}	30·00 ± 8·00^{++} (12)	1·13 ± 0·11^{++} (12)	22·85 ± 2·29^{++} (14)
Paracetamol 125 mg kg^{-1}	27·90 ± 5·30^{+} (12)	1·44 ± 0·13^{++} (12)	32·80 ± 6·48^{++} (10)
Paracetamol 250 mg kg^{-1}	19·60 ± 5·70 (12)	1·67 ± 0·10^{*++} (12)	22·79 ± 2·55^{++} (12)

Values indicate means ± s.e.m.
Figures in parentheses indicate the number of rats used in each group.
$^{*}P < 0·01$ when compared with its own control pretreated p.o. with 4% Tween 80.
$^{+}P < 0·05$, $^{++}P < 0·01$ when compared with the corresponding group given distilled water p.o. in A.

Table 4. Effects of acute paracetamol pretreatment (given s.c. 30 min before oral administration of ethanol) on ethanol-induced gastric emptying rate and gastric volume and acid level changes (rats killed 1 h after ethanol).

Pretreatment (p.o.)	Emptying rate (% of pellets expelled)	Gastric volume (mL/100 g h^{-1})	Acid content (μequiv/100 g h^{-1})
A. Distilled water 10 mL kg^{-1} p.o.			
Tween 80 4% mL kg^{-1}	59·20 ± 8·50 (15)	0·00 ± 0·00 (15)	15·25 ± 1·48 (13)
Paracetamol 125 mg kg^{-1}	56·10 ± 8·10 (11)	0·00 ± 0·00 (11)	12·96 ± 1·03 (10)
Paracetamol 250 mg kg^{-1}	25·00 ± 5·60* (12)	0·06 ± 0·03* (12)	13·41 ± 1·92 (14)
B. Ethanol 40% 10 mL kg^{-1} p.o.			
Tween 80 4% 5 mL kg^{-1}	27·10 ± 5·00^{+} (12)	0·93 ± 0·11^{+} (12)	28·70 ± 3·63^{+} (13)
Paracetamol 125 mg kg^{-1}	29·62 ± 2·71^{+} (12)	1·39 + 0·12^{*+} (12)	24·01 ± 2·77^{+} (13)
Paracetamol 250 mg kg^{-1}	34·80 ± 4·60 (11)	1·51 ± 0·13^{*+} (11)	29·70 ± 2·79^{+} (10)

Values indicate means ± s.e.m.
Figures in parentheses indicate the number of rats used in each group.
$^{*}P < 0·01$ when compared with its own control pretreated s.c. with 4% Tween 80.
$^{+}P < 0·01$ when compared with the corresponding group given distilled p.o. in A.

mucus secretion from the gastric mucosal epithelial would have an ulcer-preventing effect by lessening stomach wall friction during peristalsis, by improved buffering of acidic gastric juice and by acting as a barrier to back-diffusion of hydrogen ions. The physical property of mucus allows it to exist as an unstirred layer adhering to the mucosa, to provide a pH gradient across the epithelium at the interface between the mucus layer and the epithelial cells (Ross et al 1981; Takeuchi et al 1983). This would retard acid back-diffusion which damages the mucosal cells (Williams & Turnberg 1980). Additionally, mucus maximizes the neutralizing action of bicarbonates, in the space between the mucus gel and the epithelium, against back-diffused acid (Allen & Garner 1980), to provide a favourable microenvironment over areas of gastric damage to allow re-epithelialization (Wallace & Whittle 1986a). The importance of adherent mucus in protecting the gastroduodenal mucosa from endogenous and exogenous ulcerogenic factors has been empha-

sized in recent studies (Bell et al 1985; Wallace & Whittle 1986b) and mucus is considered to be the first line of defence. Therefore, the significant increase in mucosal mucus by paracetamol would indeed be expected to strengthen the mucosal barrier to protect the stomach from ethanol-induced damage.

Both ethanol and paracetamol delayed the gastric emptying rate by a similar degree. The significantly greater gastric volume found in ethanol-treated rats is probably mainly due to direct stimulation of the secretory cells, because it has been shown that ethanol can facilitate the release of acetylcholine at the synaptic junction (Goldstein 1983) and of gastrin from G cells (Cho et al 1987) to stimulate gastric secretion. Since the gastric emptying rate closely paralleled the volume of residual gastric secretion in paracetamol-treated rats (Tables 3 & 4), the small increase in residual gastric volume induced by the drug is likely to be due to slowed gastric emptying.

References

Allen, A., Garner, A. (1980) Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. Gut 21: 249–262

Alphin, R. S., Vokac, V. A., Gregory, R. L., Bolton, P. M., Tawes, J. W. (1977) Role of intragastric pressure, pH, and pepsin in gastric ulceration in the rat. Gastroenterology 73: 495–500

Bartle, W. R., Gupta, A. K., Lazar, J. (1986) Nonsteroidal anti-inflammatory drugs and gastrointestinal bleeding. A case-control study. Arch. Intern. Med. 146: 2365–2367

Bell, A. E., Sellers, L. A., Allen, A., Cunliffe, W. J., Morris, E. R., Ross-Murphy, S. B. (1985) Properties of gastric and duodenal mucus: effect of proteolysis, disulfide reduction, bile, acid, ethanol, and hypertonicity on mucus gel structure. Gastroenterology 88: 269–280

Cho, C. H., Ogle, C. W., Wong, S. H., Lam, S. K. (1987) Effects of ethanol and nicotine on gastrin and somatostatin release in rats. Hormone Res. 25: 113–119

Corne, S. J., Morrissey, S. M., Woods, R. J. (1974) A method for the quantitative estimation of gastric barrier mucus. J. Physiol. 242: 116p–117p

Danon, A., Assouline, G. (1979) Antiulcer activity of hypertonic solutions in the rat: possible role of prostaglandins. Eur. J. Pharmacol. 58: 425–431

Goldstein, D. B. (1983). In: Goldstein, D. B. (ed.) Pharmacology of Alcohol. 1st edn, Oxford University Press, New York, pp 3–17

Hawkey, C. J., Prichard, P. J., Somerville, K. W. (1986) Strategies for preventing aspirin-induced gastric bleeding. Scand. J. Gastroenterol. 21 (Suppl. 125): 170–173

Koo, M. W. L., Ogle, C. W., Cho, C. H. (1985) The effects of cold-restraint stress on gastric empyting in rats. Pharmacol. Biochem. Behav. 23: 969–972

Koo, M. W. L., Cho, C. H., Ogle, C. W. (1986) Effects of cold-restraint stress on gastric ulceration and motility in rats. Pharmacol. Biochem. Behav. 25: 775–779

Lanza, F. L., Royer, G. L., Nelson, R. S., Rack, M. F., Seckman, C. E., Schwartz, J. H. (1986) Effect of acetaminophen on human gastric mucosal injury caused by ibuprofen. Gut 27: 440–443

Leeling, J. L., Johnson, N. Jnr., Helms, R. J. (1981) Effect of paracetamol co-administration on aspirin-induced gastrointestinal bleeding in dogs. J. Pharm. Pharmacol. 33: 61–62

McDonald-Gibson, W. J., Collier, H. O. J. (1979) Paracetamol potentiates acetylsalicylate in inhibiting prostaglandin synthesis. Eur. J. Pharmacol. 58: 497–500

Ogle, C. W., Cho, C. H., Wong, S. H. (1985) The effect of nicotine on ethanol-induced gastric ulcers in rats. Experientia 41: 1140–1141

Pfeiffer, C. J., Lewandowski, L. G. (1971) Comparison of gastric toxicity of acetylsalicylic acid with route of administration in the rat. Arch. Int. Pharmacodyn. Ther. 190: 5–13

Robert, A. (1980) Prostaglandins and digestive diseases. Adv. Prostaglandin Thromboxane Leukotriene Res. 8: 1533–1541

Ross, I. N., Bahari, H. M. M., Turnberg, L. A. (1981) The pH gradient across mucus adherent to rat fundic mucosa in vivo and the effect of potential damaging agents. Gastroenterology 81: 713–718

Seegers, A. J. M., Jager, L. P., Van Noordwijk, J. (1979) Effects of phenacetin, paracetamol and caffeine on the erosive activity of acetylsalicylic acid in the rat stomach: dose-response relationships, time course of erosion development and effects on acid secretion. J. Pharm. Pharmacol. 31: 840–848

Takeuchi, K., Magee, D., Critchlow, J., Matthews, J., Silen, W. (1983) Studies of the pH gradient and thickness of frog gastric mucus gel. Gastroenterology 84: 331–340

Tolman, E. L., Fuller, B. L., Marinan, B. A., Capetola, R. J., Levinson, S. L., Rosenthale, M. E. (1983) Tissue selectivity and variability of effects of acetaminophen on arachidonic acid metabolism. Prostaglandins Leukotrienes Med. 12: 347–356

Van Kolfschoten, A. A., Dembinska-Kiec, A., Basista, M. (1981) Interaction between aspirin and paracetamol on the production of prostaglandins in the rat gastric mucosa. J. Pharm. Pharmacol. 33: 462–463

Van Kolfschoten, A. A., Zandberg, P., Jager, L. P., Van Noordwijk, J. (1983) Protection by paracetamol against various gastric irritants in the rat. Toxicol. Appl. Pharmacol. 69: 37–42

Wallace, J. L., Whittle, B. J. R. (1986a) The role of extracellular mucus as a protective cap over gastric mucosal damage. Scand. J. Gastroenterol. 21 (Suppl. 125): 79–84

Wallace, J. L., Whittle, B. J. R. (1986b) Role of mucus in the repair of gastric epithelial damage in the rat. Gastroenterology 91: 603–611

Williams, S. E., Turnberg, L. A. (1980) Retardation of acid diffusion by pig gastric mucus: A potential role in mucosal protection. Ibid. 79: 299–304

Wong, S. H., Ogle, C. W., Cho, C. H. (1986). The influence of chronic or acute nicotine pretreatment on ethanol-induced gastric ulceration in the rat. J. Pharm. Pharmacol. 38: 537–540