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Research Article

Paracetamol [acetaminophen]-induced gastrotoxicity: revealed by induced hyperacidity in combination with acute or chronic inflammation

K. D. Rainsford[1,2] and M. W. Whitehouse[2]*

[1] Biomedical Research Centre, Sheffield Hallam University, Sheffield, S1 1WB, UK, Tel.: +44-114-225 3006, Fax: +44-1246-583-815
[2] Therapeutics Research Unit, Department of Medicine, University of Queensland, Princess Alexandra Hospital, Woolloongabba, Qld., Australia, 4102, e-mail: whitehousemd@spin.net.au, k.d.rainsford@shu.ac.uk

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Abstract. Paracetamol is regarded as a relatively safe drug in the gastro-duodenal region of humans but recent epidemiological investigations have suggested that at high doses there may be an increased risk of ulcers and bleeding. To investigate the possibility that inflammatory conditions and gastric acidity may play a role in potentiating development of gastric mucosal injury from paracetamol in rats (as noted previously with various non-steroidal anti-inflammatory drugs) we studied the gastric irritant effects of paracetamol and some phenolic and non-phenolic analgesics and antipyretics in rats with adjuvant or collagen II induced arthritis or zymosan-induced paw inflammation and given 1.0 ml hydrochloric acid (HCl) 0.1 M and/or an i. p. injection of the cholinomimetic, acetyl-β-methyl choline chloride 5.0 mg/kg. Gastric lesions were determined 2 h after oral administration of 100 or 250 mg/kg paracetamol or at therapeutically effective doses of the phenolic or non-phenolic analgesics/antipyretics. The results showed that gastric mucosal injury occurred with all these agents when given to animals that received all treatments so indicating there is an adverse synergy of these three factors, namely: (i) intrinsic disease; (ii) hyperacidity; and (iii) vagal stimulation for rapidly promoting gastric damage, both in the fundic as well as the antral mucosa, for producing gastric damage by paracetamol, as well as the other agents. Removing one of these three predisposing factors effectively blunts/abolishes expression of this paracetamol-induced gastrotoxity in rats. These three factors, without paracetamol, did not cause significant acute gastropathy.

* *Corresponding author*

Key words: Paracetamol; Acetaminophen; Phenols; Gastric ulcer; Inflammation; Acid production; Cholinergic activation.

1. Introduction

A major practical problem frequently encountered in therapy is giving adequate pain relief but this is well-known to be bedeviled with complications; witness the recent upheaval from the withdrawal of rofecoxib (Vioxx®; Merck) – a drug presumed to have low gastrointestinal toxicity, but which unexpectedly was associated with serious cardiovascular complications (Topol, 2004, 2005).

Paracetamol (acetaminophen) has had a long history of causing low gastro-duodenal injury in both humans and normal animals (Hofteiezer et al., 1982; Ivey, 1986; Lanza et al., 1998; Prescott, 2001). However, there has been much interest recently in the question of whether this drug is likely to be associated with upper gastrointestinal (GI) damage in patients with chronic pain (e. g. Cryer, 2003; Bannwarth, 2004). Epidemiological evidence from prescription data in the UK General Practice Database has shown that while low doses of paracetamol are not associated with any significant risk for developing GI bleeding or ulcers, at doses greater than 2000 mg/d which are often used to treat rheumatic pain there is a marked increase in risk in a dose-related manner (Garcia-Rodriguez and Hernandez-Diaz, 2001). Moreover, paracetamol in association with NSAIDs was found in these studies to cause a marked increase in serious GI adverse events (e. g. bleeding ulcers) compared with that of either the NSAIDs or paracetamol alone (Garcia-Rodriguez and Hernandez-Diaz, 2001). There is also substantial data

which shows that paracetamol at high doses does produce upper GI symptoms (heartburn, abdominal pain, nausea and vomiting (Rampal et al., 2002; Bannwarth, 2004; Moore et al., 2004). The observations of Garcia-Rodriguez and Hernandez-Diaz (2001) are reinforced from a retrospective cohort study in elderly patients which showed dose-dependent increased risks of gastro-intestinal side effects from paracetamol (Rahme et al*.,* 2002). Both these studies have been questioned including the possibility of bias (Cryer, 2003; Bannwarth, 2004). There are also data from meta analysis of large scale trials comparing celecoxib with paracetamol that suggest the latter has no greater incidence of gastric adverse events compared with various doses of celecoxib (Moore et al., 2005). It seemed, therefore, imperative to establish if high dose paracetamol has the propensity to elicit GI injury under certain conditions e.,g. chronic inflammatory disease or increased acidity which are reliably reproduced in laboratory animals. Hence, this prompted us to investigate the gastric mucosal reactions to paracetamol in animals with inflammatory stress conditions and in whom gastric acid secretion was stimulated with a cholinomimetic drug given hydrochloric acid, both conditions of which are known to exacerbate injury from NSAIDs and which are known to be important in development of NSAID injury (Rainsford and Brune, 1976; Rainsford, 2004). We have previously shown these stressors can markedly enhance the gastro-ulcerogenic effects of most non-steroidal anti-inflammatory drugs (NSAIDs), even those with the reputation for being the mildest irritants (Rainsford, 1987, 1989, 1999).

We show here that a drug traditionally thought to have low gastro-duodenal injury, paracetamol (acetaminophen), induces gastric toxicity in rats exposed to acute or chronic inflammatory insult during which a wide range of inflammatory mediators are released but only where gastric acidity is stimulated.

2. Methods

2.1. Induction of acute inflammation

Carrageenan 0.5 mg in 0.1 ml physiological saline was injected in the hindpaws of rats using procedures as described (Rainsford and Whitehouse, 1977). The acute anti-inflammatory activity was determined at 2 and 24 h by measurement of the paw thickness using an engineer's micrometer. Zymosan-induced rear paw oedema was established 48 h previously be injecting 0.5 mg zymosan-A (Sigma), dispersed in 0.2 ml saline. The paw swellings were determined as described above.

2.2. Induction of chronic inflammation

Adjuvant-induced polyarthritis was established in female Dark Agouti rats by tailbase s.c. inoculation of 600 µg heat-killed and delipidated *Mycobacterium tuberculosis* (hominis) dispersed in 0.1 ml squalane (Whitehouse, 1988). Collagen-induced arthritis was established in female Wistar rats with a single inoculation of 250 µl emulsion given intradermally (Bakharevski et al*.,* 1988) into the tailbase (in five divided doses) containing 125 µg collagen in 125 µl 10 mM acetic acid and 125 µl Freund's incomplete adjuvant (Sigma Chem. Co., St Louis, MO). Polyarthritic animals were used 13–15 days later depending on disease severity.

2.3. Gastric mucosal injury

Paracetamol was given p.o. at 250 and 100 mg/kg finely dispersed in water to which a few drops of Tween were added. The cholinomimetic, betamethacholine (acetyl-β-methyl choline) chloride (Sigma; abbreviated MC) which markedly sensitizes the gastric mucosa to injury from NSAIDs but is not itself injurious to the mucosa (Rainsford, 1987, 1999) was administered at a dose of 8 mg/kg in 1 ml 0.9 % saline i.p. after oral dosing of paracetamol with or without 1 ml I00 mM hydrochloric acid. Animals were sacrificed three hours later, their stomachs removed, opened and rinsed with water and then inspected for macroscopic haemorrhagic lesions. The macroscopic appearance of the stomachs was recorded by digital photography. The number and severity of gastric lesions and the gastric swelling or distension (an indicator of pylorospasm and reactions to irritants e.g. by release of histamine) were recorded. The gastric Lesion Index was compiled from the number of lesions multiplied by the severity – this being a composite measure of the approximate area of the mucosal lesions.

2.4. Antipyretic activity

To determine the surrogate inhibitory effects of paracetamol and other phenols on the production of prostaglandin (PG)E_2 in vivo studies were performed to establish their antipyretic activity. The test compounds were given to Wistar rats with pre-established fever induced by s.c. injection of Brewer's yeast 1.2 g/kg given 9 h previously. The temperature changes were measured rectally using a clinical thermometer.

2.5. Statistical Analysis

Gastric lesion data are presented in the form of descriptive tests giving value for the means, confidence limits of the mean and medians as transformed parametric data. To explore the most satisfactory and unbiased assessment of statistical significance non-parametric statistical tests were performed on the data using the randomization, Mann-Whitney and Kolmogorov-Smirnov tests. While all three tests gave similar results the Kolmogorov-Smirnov test was found the most reliable and the data were therefore analyzed for significance using this test; a probability level of 0.05 was taken as being the level below which there was statistical significance.

2.6 Ethical approval

The procedures employed in these studies were approved by the University of Queensland Animal Ethics Committee No. 5.

3. Results

The data in Table 1 shows that paracetamol 100 or 250 mg/kg p.o. caused development of appreciable gastric mucosal injury when administered to rats with adjuvant- or collagen-induced arthritis or zymosan-induced hind-paw oedema that additionally had received 0.1 M HCl p.o. and the cholinergic agent, acetyl β-methyl choline chloride [MC] 5 mg/kg i.p. at the same time. The number of lesions was significantly ($p < 0.05$, Kolmogorov-Smirnov test) greater following these combined treatments than when the animals were given paracetamol and either the acid or cholinometic treatments alone or in the absence of both these acidifying agents. The extent of gastric mucosal injury was paralleled in the gastric

Table 1. Gastric Ulcerogenicity of Paracetamol in Acid- and Cholinomimetic- stimulated Rats with Inflammatory Conditions

Dose Paracetamol mg/kg p.o.	Treatments MC	HCI	N	*No. of gastric lesions mean (95% CI) (median)	Statistical significance K-S D	P	Lesion Index	Gastric Swelling (mean)
A. Adjuvant arthritis								
(1) none (control)	+	+	7	7.3 (3.2 - 11.5) (7.5)			15,7	2,7
(2) 100	+	+	15	62.2 (51.7 - 72.7) (64.2)	0,83	0,003 c.f.con	78,1	2,9
(3) 250	+	+	15	62.9 (52.5 - 73.4) (63.0)	1	0,001 c.f. con	178	2,9
(4) 250	+	-	12	3.75 (1.5 - 6.05) (3.0)	1,583	0.08 c.f con	3,8	1
					1	<0.001 c.f #3		
(5) 250	-	+	7	1.6 (-1.5 - 4.7) (0)	0,8	0.026 c.f. con	2,4	0,6
					1	<0.001 c.f. #3		
(6) 250	-	-	4	1.0 () (1.0)			1,3	1,3
B. Collagen II arthritis								
(1) none (control)	+	+	15	10.7 (6.0 - 15.4) (8.0)			19,7	2,2
(2) 100	+	+	4	57.8 (29.3 - 86.2) (60.5)	0,87	0.006 c.f. con	173	2,3
(3) 250	+	+	11	51.3 (35.5 - 67.0) (53.0)	0,818	<0.001 c.f. con	149	3,6
(4) 250	-	+	5	0.2 (-0.19 - -0.52) (0)	0,933	<0.001 c.f. con	0,2	1,4
					1	<0.001 c.f. #3		
(5) 250	+	-	5	1.4 (-0.3 - 3.1) (0)	0,867	<0.002 c.f. con	2,8	2,2
					1	<0.001 c.f. #3		
C. Zymosan-oedema								
(1) none (control)	+	+	6	5.7(0.8-10.5) (4.5)			5,7	2,2
(2) 100	+	+	5	29.4 (21.1-37.7) (24.0)	1	0.002 c.f. con	54	2,3
(3) 250	+	+	4	63.8 (52.1-75.4) (69.0)	1	0.005 c.f. con	197	3,6
(4) 250	+	-	8	4.6 (1.8-7.3) (3.0)	0,37	>0.05 c.f. con	4,2	2,2
					1	0.003 c.f. #3		
(5) 250	-	+	3	3.3 (2.7-4.0) (3.0)	0,67	>0.05 c.f. con	**v**3,3	1,4
					1	0.021 c.f. #3		

Paracetamol was given p.o. at 250 and 100 mg/kg
β-MC = Acetyl – β-methacholine hydrochloride at 8 mg/kg i.p. in saline: a cholinomimetic drug, that enhances the gastrotoxicity of all established NSAIDs in rats and mice, with little effect in control (untreated) animals) (Rainsford, 1999), HCl = hydrochloric acid, 133 mM p.o., *mean number macroscopic haemorrhagic lesions in groups of 2 to 4 rats fasted overnight but given drinking water. Severity, scored 1+ to 3+.
Bleeding was also evident in the antrum of some paracetamol treated rats given the combined treatments.

Lesion Indices being greater in rats given paracetamol with HCl and MC treatments compared with those that had not been given the acidifying treatments (Table 1). Rats that had been given paracetamol 100 or 250 mg/kg alone did not develop significantly greater mucosal injury compared with control animals that received H_20 alone; in both groups only a few small lesions were apparent (data not shown).

The figures show the appearance of the gastric mucosa from adjuvant arthritic rats given paracetamol 100mg/kg with MC and HCl (Fig. 1A), MC and HCl but no paracetamol (Fig. 1B) or control animals given H_20 alone (Fig. 1C). Extensive haemorrhagic mucosal damage is evident in the animals given paracetamol with MC and HCl (Fig. 1A), whereas only a few lesions were observed in those given MC and HCl but no paracetamol (Fig. 1B), while there was no mucosal damage in the control animals. The appearance of the haemorrhagic lesions in the paracetamol with MC-HCl treated animals was identical to that seen in animals given aspirin or other NSAIDs either alone or with the acidifying agents.

Similar increase in the gastric mucosal injury was observed in arthritic rats or those with zymosan paw swelling given HCl and MC that were dosed orally with a range of phenolic antipyretics or analogues. Thus, administration of salicylic acid 100 or 140 mg/kg, methyl salicylate 100 mg/kg, saligenin (salicyl alcohol) 200 mg/kg, methyl paraben (isomer of methyl salicylate) 100 or 250 mg/kg, butyl paraben 130 mg/kg, propyl paraben 120 mg/kg, 200 mg/kg, eugenol 270 mg/kg, guaiacol 100 mg/kg, dipyrone 290 mg/kg or amidopyrine 150 or 190 mg/kg caused a marked increase in mucosal lesions in animals given these combined treatments, i.e. acidifying agents in animals with acute (zymosan) or chronic (arthritis) treatments compared with those animals that had not received either MC or HCl (data not shown). These results show that the enhancement of gastric ulcerogenicity is a property of phenolic as well as non-phenolic (antipyrine and dipyrone) anti-pyretics or analgesics.

To establish if the dose range of drugs/agents used in the abovementioned tests for gastric ulcerogenicity had anti-inflammatory or antipyretic activities assays were performed in the standard carrageenan-paw oedema and yeast-induced pyresis assays respectively. The results in Table 2 show that the phenolic salicylate compounds all exhibited anti-oedemic effects while paracetamol (which is not an anti-inflammatory agents) as expected was ineffective. These phenolic compounds as well as paracetamol all were found to have to have anti-pyretic activity at doses within the range of those em-

Effects of paracetamol 100 mg/kg p.o. given with 1 ml 0.1M HCl and acetyl- -methyl choline or methacholine (MC) 8 mg/kg i.p. to fasted adjuvant arthritic rats. The macro-photographs were recorded digitally and are not enhanced or edited.

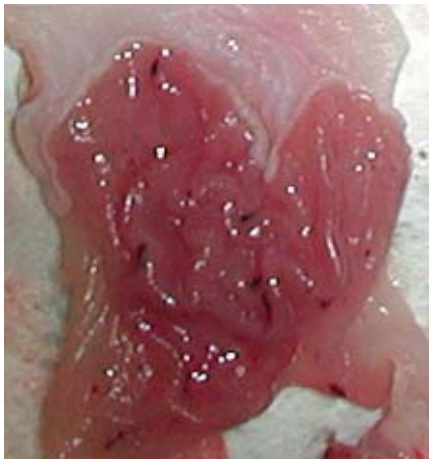

Fig. 1A. Appearance of the fundic and antral mucosa of an arthritic rat given 100 mg/kg paracetamol, 1 ml of 0.1 M HCl and MC. Note dark patches of haemorrhagic lesions.

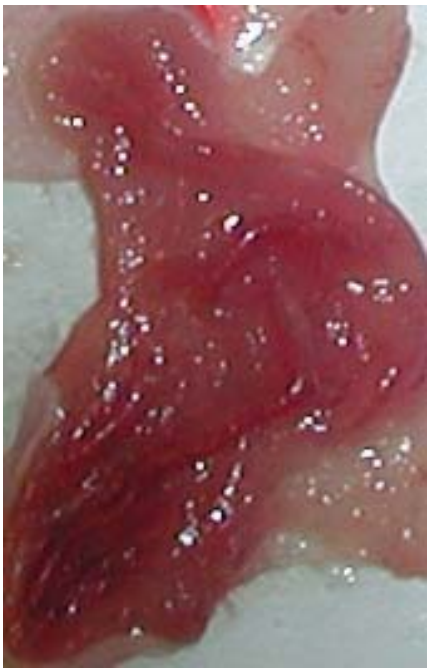

Fig. 1B. Gastric mucosa of an arthritic rat given MC and HCl.

Fig. 1C. Gastric mucosa of a control arthritic rat given 1 ml H_2O.

Table 2. Acute Anti-Inflammatory activity in the carrageenan Paw Oedema

Wistar rats given 0.5 mg carrageenan in 0.1 ml saline in each rear paw. Test compounds administered orally 45 min previously. Paw swelling measured with a micrometer. N = 3 rats/group

Compound	mg/kg	Mean percentage inhibition 2 h	24 h
Paracetamol	250	09	12
Salicylic acid	140	66	
Sodium salicylate	150	47	24
Saligenin	300	25	–80
Saligenin	150	17	–140 (!)
Aspirin	150	60	–105
Celecoxib	25	37	06
Ketoprofen	10	80	

Table 3. Anti-pyretic activities

Test compounds given orally to female Wistar rats with pre-established fever generated by s.c. injection brewer's yeast (1.2 g/kg) 9 h previously. Temperatures measured rectally. N = 3 rats/group

Test compound	mg/kg	Δ T (°C) 1 h	Δ T (°C) 2.2 h
Paracetamol	100	–1.1	–1.8
Eugenol	100	–1.7	–0.3
Guaiacol	100	–1.4	–0.2
Saligenin	200	–2.6	–2.5
Saligenin	150	–2.0	–1.9
Saligenin	75	–1.4	–1.1
Ketorolac (Tris salt)	25	–1.0	–1.2
Celecoxib	25	–0.9	–1.0
Aspirin	150	–1.0	–0.6

Ketorolac was gastrotoxic at 2.5 h (no HCL, no MC) in these unfasted rats. Initial fever temperature was 39.4 °C.

ployed in the gastric ulcer assays. Thus, the dose range chosen in the ulcer assays was within that for therapeutic effects.

4. Discussion and conclusions

The results show clearly that gastric ulcerogenicity of paracetamol and other phenolic and non-phenolic analgesic/antipyretic agents is expressed when these drugs are given to rats with acute or chronic inflammation that simultaneously have enhanced gastric acidity in the form of HCl and MC treatments.

Recently, the issue has focussed on the question whether paracetamol is likely to cause gastric damage in patients with chronic pain? The consensus has generally been that this drug is not likely to provoke major gastric problems (Prescott, 2001), resembling the well-described NSAID-gastropathy evidenced by gastro-duodenal ulcers and persistent bleeding (Garcia-Rodriguez and Hernandez-Diaz, 2001).

In laboratory animals paracetamol or dipyrone (metamizol) given orally at modest doses even under subchronic conditions do not cause GI mucosal damage or inflammation in normal animals (Sanchez et al., 2002). Inhibition of gastric mucosal prostaglandin production has

long been recognized as a major factor in the development of gastric mucosal injury from NSAIDs (Rainsford, 2004). Paracetamol is known under some conditions to inhibit cyclo-oxygenase (COX)-1 and COX-2) in whole blood monocytes or macrophages (Brune et al., 1981a; Sciulli et al., 2003) although it does not appear to inhibit production of gastric prostaglandins (Cryer and Feldman, 1998). The lack of effects on prostaglandin production may be due to the relatively limited uptake of the drug into gastric mucosal cells (Brune et al., 1981b). Given stimulation of acid production the systemic inhibition of prostaglandin production may be sufficient to cause alterations in vascular functions that may predispose development of gastric damage when sufficiently high doses of the drug are taken.

Here we propose the caveat that, under some circumstances, paracetamol is no different from other cyclooxygenase inhibitors (Garcia-Rodriguez and Hernandez-Diaz, 2001) and may indeed elicit gastric distress. These circumstances may include a) hyperacidity in the stomach e. g. nocturnal rise in the secretion of hydrochloric acid (Shih et al., 2003), and b) supervening stresses beyond those of the pain-eliciting arthropathy e. g. activation of the vagus to intensify gastric secretion (Rainsford, 1987, 1989, 1999).

Accordingly, we propose that non-arthritic 'stressors', often overlooked in a cursory examination, might ultimately determine the patient's susceptibility to a 'paracetamol [acetaminophen]-gastropathy'. If this condition is readily provoked in rats, might it not also arise under some circumstances in humans? It seems to be intrinsic to the paracetamol, rather than the stressors which unmask it e. g. disease status, gastric acidity, or the neural tone of the stomach.

The hyperacidity, stress and inflammatory disease-related factors, here shown to interact with paracetamol, may possibly contribute to the two-fold increase in the risk of serious upper gastro-intestinal complications at daily doses of >2 g/d of this analgesic (Garcia-Rodriguez and Hernandez-Diaz, 2001; Rahme et al., 2002).

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