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Interaction between aspirin and paracetamol on the production of prostaglandins in the rat gastric mucosa

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Recently Seegers et al (1980a) suggested that reduction of the gastric erosive activity of aspirin by paracetamol in the rat is due to stimulation of endogenous production of prostaglandins. This hypothesis was based on the observations of Robak et al (1978) and McDonald-Gibson et al (1979) that paracetamol could stimulate the prostaglandin-synthetase system of bovine seminal vesicles in vitro. The latter authors also observed that stimulation by paracetamol renders that enzyme system more vulnerable to the inhibitory action of aspirin. It is well known that the prostaglandin-synthetase systems in different organs show different sensitivities to peripheral analgesics. The aim of the present study was to investigate interactions between aspirin and paracetamol in vivo on the prostaglandin biosynthesis in the rat gastric mucosa under circumstances comparable with those in which the interactions on gastric damage were observed (Seegers et al 1979).

Wistar rats, 160–200 g, were starved for 36 h while in cages with a metal grid (to avoid coprophagy) and allowed free access to water. Aspirin and paracetamol were suspended in 4% Tween-20 and administered orally in a volume of 0·5 ml/100 g. Control rats received a similar volume of 4% Tween-20 solution. The dosages of both aspirin and paracetamol were 250 mg kg^{-1}, because previously (Seegers et al 1979) this dose of aspirin caused considerable gastric damage that was reduced significantly by the same dose of paracetamol.

Prostaglandin production in the glandular mucosa was measured 0·5 h after drug administration, a time at which the aspirin-induced erosion-foculi are not inhibited by paracetamol, and 5 h and 17 h after administration when the erosion scores are reduced to control values by paracetamol (Seegers et al 1979). At these times the rats were killed, the stomach was removed, opened along the lesser curvature and rinsed in ice-cold 0·9% NaCl. A modification of the method of Whittle (1978) and Whittle et al (1980) was used to estimate prostacyclin production. Strips (20–100 mg) of gastric mucosa were removed from the muscle layer; 100 mg was cut into small pieces, washed in buffer (Tris 50 mM; pH 9·5) and incubated in 0·2 ml of buffer by vortex mixing (1 min, 22 °C). After centrifugation for 1 min, aliquots (5–50 μl) of the supernatant were immediately tested for their ability to inhibit rabbit platelet aggregation, induced with ADP (2 μM). In this bioassay of prostacyclin-like activity, authentic PGI_2 was used as a reference. Timelapse between death of the animal and start of the platelet assay was less than 10 min. The remainder of the supernatant was boiled for 1 min and assayed for prostaglandin E_2-like activity on the superfused rat stomach strip against authentic PGE_2 (Vane 1969). PGE_2 and PGI_2 were donated by the Upjohn Company. These PG's were chosen as references because Pace-Asciak & Rangaraj (1977) showed PGE_2 and 6-oxo-$PGF_{1\alpha}$, the stable breakdown product of PGI_2, to be the major products of arachidonic acid metabolism in the rat stomach.

All data are given as the means with their standard errors; differences were tested with Student's *t*-test; probability levels less than 0·05 were taken as significant. Comparison of vehicle-treated with untreated rats at different times revealed no influence of Tween-20 on prostaglandin production. Therefore values of vehicle-treated and of untreated rats were pooled and taken as control values.

The results (Table 1) show that at all sampling times aspirin reduced the formation of prostacyclin-like activity to levels which were not detectable (<20 ng g^{-1} tissue). Paracetamol alone had no influence on prostacyclin formation at any time. The combination of paracetamol with aspirin inhibited the production of prostacyclin-like activity below the detection limit, just as aspirin alone. Gas chromatography-mass spectra

Table 1. The influence of aspirin (250 mg kg^{-1}), paracetamol (250 mg kg^{-1}) and the combination of the two drugs on the production of PGI_2- and PGE_2-like activity in the rat gastric mucosa, 0·5 h, 5 h and 17 h after oral administration.

Treatment	Time after admin. (h)	PGI_2 ng g^{-1} tissue*	n	PGE_2 ng g^{-1} tissue*	n
Control	—	214 ± 36	13	435 ± 54	19
Aspirin	0·5	<20†	5	67 ± 18†	7
Paracetamol	0·5	235 ± 39	7	428 ± 84	7
Aspirin + paracetamol	0·5	<20†	5	106 ± 24†	6
Aspirin	5	<20†	5	45 ± 5†	6
Paracetamol	5	291 ± 53	8	743 ± 73†	7
Aspirin + paracetamol	5	<20†	5	74 ± 17†	6
Aspirin	17	<20†	8	134 ± 40†	8
Paracetamol	17	282 ± 41	6	155 ± 25†	6
Aspirin + paracetamol	17	<20†	8	121 ± 48†	8

* Mean ± s.e.m.
n = number of animals.
† significantly different from control value; $P < 0·05$.

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(g.c.-m.s.) analysis of a sample from untreated rats showed that the mucosa contained about 500 ng g^{-1} tissue 6-oxo-$PGF_{1\alpha}$. Comparison of this amount with the values obtained with the bioassay indicates that the prostacyclin-like activity assayed by platelet aggregation is largely, if not solely, due to PGI_2. The apparent discrepancy between the levels of PGI_2 detected by bioassay (214 ng g^{-1}) and the levels of the breakdown product 6-oxo-$PGF_{1\alpha}$ (500 ng g^{-1}) detected by g.c. -m.s. could arise from degradation of PGI_2 before and during incubation and sampling.

With regard to the production of PGE_2-like activity, the results in Table 1 show that aspirin has an inhibitory effect which was not altered by concurrent administration of paracetamol. Given alone, paracetamol had no effect on the PGE_2-like activity at 0·5 h but increased it at 5 h. However after 17 h a decrease was observed.

The ratio between the amounts of PGI_2 and PGE_2 produced by control rats is different from that observed by Pace-Asciak & Rangaraj (1977). The relatively high amounts of PGE_2-like material in our ex vivo experiments could reflect methodological as well as rat strain differences. The latter point is illustrated by the fact that Whittle (1978), using the same method, found about half the PGI_2-like activity reported here.

It can be concluded that although a stimulatory effect of paracetamol on the PG biosynthesis system of the glandular mucosa of the rat stomach was demonstrable, paracetamol did not alter the inhibitory activity of aspirin. It is not impossible that some PGI_2 is still being produced after administration of aspirin and that there is some stimulation of the synthesis of PGI_2 by paracetamol in the presence of aspirin which cannot be detected by the method used. However, it is questionable whether a stimulation of such a marginal prostacyclin production could yield levels of PGI_2 that could protect the gastric mucosa against aspirin damage. Therefore it seems unlikely that the hypothesis of Seegers et al (1980a) explains the protective action of paracetamol against the erosive activity of aspirin in the glandular mucosa of the rat stomach.

Our observation that paracetamol alone stimulated PGE_2 but not PGI_2 production at 5 h could be explained as a stimulation of the generation of the endoperoxide PGH_2 (Kuehl et al 1977) and/or as a stimulation of PGE_2-isomerase (Yamamoto et al 1980). Kuehl et al (1977) reasoned that drugs like paracetamol could stimulate the generation of prostaglandins of the E- and F-type by facilitating the conversion of PGG_2 to PGH_2. During this conversion noxious free radicals are produced which inhibit the reaction; paracetamol may act as a scavenger of these free radicals and may promote the generation of PGH_2. Furthermore the fact that only PGE_2 production was increased suggests a stimulation of PGE_2-isomerase as found by Cottee et al (1977) with reduced glutathion. Our data however do not allow us to discern between these two possible mechanisms of action.

The inhibition of the production of PGE_2-like activity by paracetamol alone 17 h after administration is puzzling. The half-life of paracetamol in rats is about 0·6 h (Seegers et al 1980b) indicating that at 17 h all paracetamol (and probably its metabolites) had disappeared. The inhibitory effect might be a rebound effect resulting from the stimulation in the presence of paracetamol.

The ability of paracetamol to scavenge free radicals could be an alternative explanation for its protective activity against gastric damage induced with aspirin. According to Hamberg & Samuelsson (1974) inhibitors of cyclo-oxygenase like aspirin can give rise to stimulation of alternative pathways of lipoxygenation of arachidonic acid resulting in lipid peroxides and free radicals. These products could contribute to gastric damage. Reduction of these peroxides and scavenging of free radicals by paracetamol might explain the antagonistic interaction between paracetamol and aspirin on gastric damage in rats.

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