Effect of ethanol on eicosanoid synthesis by human gastric and colonic mucosal pieces

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1 Human gastric and colonic mucosa obtained at operation was cut into small pieces and incubated with different concentrations of ethanol.

2 Ethanol (5–40%) caused a concentration-dependent increase in the amounts of prostaglandin E (PGE), thromboxane B₂ (TXB₂), 6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) and (up to 20% ethanol) leukotriene C₄/D₄ (LTC₄/D₄) in incubates of mucosal pieces from either region.

3 Higher concentrations of ethanol usually caused small increases or reductions of eicosanoid levels; gastric mucosal PGE and 6-keto-PGF_{1α} were still increased by 100% ethanol, whereas TXB₂ was unaltered, and LTC₄/D₄ was reduced. With the colonic mucosa, 100% ethanol increased PGE but reduced the other eicosanoids.

4 Gastric mucosal pieces incubated in water or phosphate buffer yielded generally similar amounts of eicosanoids. However, colonic mucosa yielded more when incubated in water, possibly indicating a greater sensitivity to osmotic damage. This difference between the two regions is consistent with the ability of the gastric mucosa to resist damage by water on its epithelial surface.

Introduction

Some natural cyclo-oxygenase products such as prostaglandin E₂ (PGE₂) and PGI₂, and synthetic analogues such as 16,16-dimethyl PGE₂ and misoprostol, protect the gastrointestinal mucosa against damage (Scratcherd, 1987; Watkinson & Akbar, 1987; Boughton-Smith & Whittle, 1988a). In contrast, 5-lipoxygenase products, particularly leukotriene C₄ (LTC₄), may mediate damage and inflammation in the gastric mucosa (Wallace & Whittle, 1985; Boughton-Smith & Whittle, 1988a) and colonic mucosa (Rampton & Hawkey, 1984; Sharon & Stenson, 1984; Boughton-Smith *et al.*, 1988; Wallace *et al.*, 1989).

Ethanol caused a concentration-dependent increase in the *ex vivo* or *in vitro* release of LTC₄ from the rat gastric mucosa (Peskar *et al.*, 1986; 1988; Boughton-Smith & Whittle, 1988a,b); prostaglandin formation was not significantly affected (Boughton-Smith & Whittle, 1988a; Peskar *et al.*, 1988). Inhibition of gastric LTC₄ formation by compounds such as sodium salicylate (Peskar *et al.*, 1988), 16,16-dimethyl PGE₂ (Boughton-Smith & Whittle, 1988a), cysteamine or diethyl maleate (Lange *et al.*, 1987), carbenoxolone or nordihydroguaiaretic acid, protected the rat gastric mucosa against ethanol-induced damage (Peskar *et al.*, 1986); again prostaglandin release was not significantly affected (Boughton-Smith & Whittle, 1988a; Peskar *et al.*, 1988).

In another recent study (Boughton-Smith & Whittle, 1988b), the selective 5-lipoxygenase inhibitors BW A4C and BW A137C, at doses that almost completely inhibited rat mucosal synthesis of LTB₄ or LTC₄, did not reduce the gastric mucosal damage by ethanol. In contrast, pretreatment with the less specific lipoxygenase/cyclo-oxygenase inhibitor BW 755C did reduce rat gastric mucosal damage by ethanol (Wallace & Whittle, 1985). Thus the relationship between ethanol-induced gastric mucosal damage and eicosanoid formation is unclear. Furthermore, these studies were mainly in the rat, and their relevance to man has not been determined. We now describe the effect of different ethanol concentrations

on the eicosanoid accumulation in incubates of human gastric and colonic mucosal pieces.

Methods

Human gastric and colonic mucosa

Gastric and colonic tissue were taken at least 5 cm from any macroscopically detected lesions in surgical specimens removed for benign or malignant disease. It was transported to the laboratory in 154 mM NaCl at ambient temperature within 30 min of removal, and on arrival it was transferred to ice-cold phosphate buffered saline pH 7.4 (PBS). The layer of mucosa/submucosa was carefully cut off from the underlying muscle while the tissue was bathed in PBS. As far as we know, the patients had not recently consumed drugs that affect eicosanoid synthesis.

Mucosal incubation

Each sample of mucosa/submucosa was rinsed in PBS, cut into pieces 3–5 mm², and washed again with PBS which was then drained off. Carefully weighed samples of 100 ± 5 mg were pre-incubated in PBS (1 ml, 0°C, 15–30 min) which was then drained off, and the tissues were transferred to tubes containing 1 ml of either PBS, distilled water or different concentrations of ethanol (5, 10, 20, 40, 80 and 100% in distilled water) for 5 min. The tissues were then taken out, rinsed well in PBS, blotted gently on filter paper and then transferred to tubes containing 1 ml of fresh ice-cold PBS for another 5–10 min.

This pre-incubation fluid, containing any released prostanooids, was discarded and the fluid was again replaced by 1 ml of fresh PBS alone. After further incubation at 37°C for 30 min, the PBS containing the released eicosanoids was removed and stored at –20°C until assayed.

Radioimmunoassays

The method used is based on that of Jaffe & Behrman (1974) and suitable dilutions of antisera and tritiated standards were

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Table 1 Effect of different concentrations of ethanol on the accumulation of endogenous eicosanoids in incubates of human gastric mucosal pieces (30 min incubation)

Incubation fluid	% difference from the water control			
	PGE	TXB ₂	6-keto-PGF _{1α}	LTC ₄ /D ₄
Buffer (PBS)	4.4 ± 27.7	-21.4 ± 11.0	0.9 ± 11.7	-5.2 ± 17.2
Ethanol (%)				
5	21.7 ± 9.9	42.3 ± 26.1	46.9 ± 34.5	18.6 ± 26.7
10	57.0 ± 41.1	110.4 ± 50.1	74.8 ± 29.5 ^a	149.3 ± 58.5
20	129.7 ± 43.2 ^c	117.7 ± 22.7 ^c	83.7 ± 40.9	242.2 ± 60.2 ^c
40	355.5 ± 114.7 ^c	319.3 ± 90.3 ^c	159.2 ± 59.4 ^b	57.0 ± 18.8 ^a
80	471.5 ± 152.5 ^c	66.4 ± 55.5	137.7 ± 30.2 ^c	-24.6 ± 14.7
100	293.0 ± 61.7 ^c	0.3 ± 33.0	111.3 ± 37.7 ^a	-66.7 ± 9.8 ^a

The results are mean ± s.e.mean expressed as the % difference from the amounts in incubates with water ($n = 6$), and analysed by Student's *t* test for paired data. ^a $P < 0.05$, ^b $P < 0.02$, ^c $P < 0.01$, compared with the water controls.

Amounts of eicosanoids (ng g^{-1} wet weight 30 min⁻¹) in the water control: PGE 120.0 ± 17.7, TXB₂ 57.7 ± 14.3, 6-keto-PGF_{1α} 107.8 ± 16.8 and LTC₄ 7.0 ± 2.0

used. Assay sensitivities were 20 pg for prostanoids and 50 pg for LTC₄. The intra- and inter-assay coefficients of variation were 5–9% and 10–11% depending on the substance measured. All assays were in duplicate.

Tritiated prostanoids were purchased from Amersham Radiochemical Centre. The % cross-reactions of the antisera used were: PGE antibody (ICN): PGE₂ 100; PGE₁ 240; PGA₁ 10; PGA₂ 8; PGF_{1α} 3.2; PGF_{2α} 4.2; PGB₁ 0.7; PGB₂ 0.9. 6-keto-PGF_{1α} antibody (Wellcome): PGF_{2α} 0.84; PGE₂ 0.1; TXB₂ 0.02. TXB₂ antibody (Wellcome): PGF_{2α} 0.11; PGE₂ 0.008; 6-keto-PGF_{1α} 0.01.

Tritiated leukotriene C₄ was purchased from New England Nuclear. The % cross-reactions of the LTC₄/D₄ antiserum, kindly provided by Dr J. Zakrzewski, were: LTC₄ 100; LTD₄ 29.4; LTE₄ 0.7; LTB₄ 0.05; PGE <0.05; arachidonic acid <0.05.

As the PGE antiserum does not distinguish between PGE₁ and PGE₂, the measurements are expressed as PGE. Since the LTC antiserum distinguishes poorly between LTC₄ and LTD₄, the measurements are expressed as LTC₄/D₄. Because the results reflect only the interaction of released material with antisera, the measurements should really be expressed as, for example, PGE-like. However, for simplicity we have referred to them as the authentic substances. Evidence that these released products are prostanoids and leukotrienes is provided by experiments using the same antisera, where indomethacin inhibited prostanoid formation in human gastric mucosa/submucosa (Tavares *et al.*, 1987), and the cyclo-oxygenase/lipoxygenase inhibitor BW755C blocked eicosanoid formation in human colon mucosa/submucosa (unpublished).

Most of the values are given to 3 significant figures; they are shown in the text as the means, and the s.e.mean are given

in the Tables. The results were analysed statistically by Student's *t* test for paired data (2-tailed), using the groups treated with distilled water (the solvent for ethanol) as the controls unless otherwise stated.

Results

Gastric mucosal prostanoids

Amounts of gastric mucosal prostanoids in the water incubates of gastric mucosa/submucosa (control group, ng g^{-1} 30 min⁻¹) were: PGE 120; TXB₂ 57.7; 6-keto-PGF_{1α} 108 ($n = 6$). Incubation in PBS instead of water made little or no difference to the prostanoid yield (PGE, +4% of the amount in the water incubates, $P < 0.4$; TXB₂ -21%, $P < 0.2$; 6-keto-PGF_{1α}, +3%, $P < 0.6$).

As shown in Table 1, ethanol (5–40%) induced a concentration-related increase in the prostanoid contents of the incubates. The PGE yield rose further with 80% ethanol, but the increase in the other prostanoids tended to be less than when 40% ethanol was used. With 100% ethanol the amounts of both PGE and 6-keto-PGF_{1α} were still higher than with water, whereas TXB₂ was almost identical to the control.

Gastric mucosal leukotrienes

Incubates of gastric mucosa/submucosa in water contained 7.0 ng g^{-1} wet weight 30 min⁻¹ of LTC₄/D₄, compared with

Table 2 Effect of different concentrations of ethanol on the accumulation of endogenous eicosanoids in incubates of human colonic mucosal pieces (30 min incubation)

Incubation fluid	% difference from the water control			
	PGE	TXB ₂	6-keto-PGF _{1α}	LTC ₄ /D ₄
Buffer (PBS)	-33.0 ± 13.8	-27.3 ± 3.9 ^c	-42.3 ± 7.1 ^c	-29.6 ± 8.9 ^a
Ethanol (%)				
5	21.2 ± 15.4	22.8 ± 18.3	-10.0 ± 8.8	41.9 ± 18.2
10	31.0 ± 11.9 ^a	14.8 ± 11.9	-4.0 ± 13.0	55.7 ± 19.4 ^a
20	95.8 ± 16.4 ^c	85.2 ± 24.0 ^a	30.7 ± 13.6	160.0 ± 59.7 ^b
40	215.7 ± 63.3 ^c	140.5 ± 29.0 ^a	75.8 ± 31.0 ^c	66.6 ± 44.8
80	295.7 ± 68.7 ^c	14.0 ± 13.1	16.0 ± 18.6	-29.1 ± 17.1
100	187.8 ± 56.1 ^b	-44.2 ± 13.2 ^a	-48.5 ± 15.6 ^a	-40.3 ± 13.7

The results are shown as means ± s.e.mean, and expressed as % difference from the water control ($n = 6$). *P* values (Student's *t* test for paired data): ^a $P < 0.05$, ^b $P < 0.02$, ^c $P < 0.01$, compared with the water incubates.

Amounts of eicosanoids (ng g^{-1} wet weight 30 min⁻¹) in the water incubates were: PGE 89.1 ± 15.1, TXB₂ 51.4 ± 11.7, 6-keto-PGF_{1α} 137.4 ± 35.6 and LTC₄/D₄ 6.3 ± 1.1

6.6 ng g⁻¹ in PBS ($P < 0.8$). Ethanol had a biphasic effect. With 5–20% there was a concentration-related increase in the mean amounts of LTC₄/D₄, whereas 40% ethanol caused a smaller increase than 10 or 20%, and 100% ethanol was inhibitory (Table 1).

Colonic mucosal prostanoids

The mean amounts of prostanoids in the aqueous incubates of colonic mucosa/submucosa were (ng g⁻¹ 30 min⁻¹): PGE 89; TXB₂ 51; 6-keto-PGF_{1α} 137 ($n = 6$). Amounts in the PBS incubates were lower (PGE by 33%, $P < 0.1$; TXB₂ 27%, $P < 0.01$; 6-keto-PGF_{1α} by 42%, $P < 0.01$).

With ethanol (5–40%) there was a concentration-related increase in the prostanoid accumulation (Table 2). At 80% ethanol the PGE accumulation increased further, whereas the mean amounts of TXB₂ and 6-keto-PGF_{1α} were little different from the controls. With 100% ethanol PGE was still increased, whereas the amounts of TXB₂ and 6-keto-PGF_{1α} were less than controls, showing a concentration-dependent biphasic effect of ethanol.

Colonic mucosal leukotriene formation

Aqueous incubates of colonic mucosa/submucosa contained a mean amount of 6.3 ng g⁻¹ wet weight 30 min⁻¹ of LTC₄/D₄, whereas the PBS incubates contained about 30% less (4.4 ng, $P < 0.05$). With 5–20% ethanol there was a concentration-related stimulation of the LTC₄/D₄ yield, but 80 and 100% ethanol tended to be inhibitory (Table 2).

Discussion

The amounts of eicosanoids in incubates of human gastric mucosa were similar when water or phosphate buffer were used. With colonic mucosa, in contrast, the water resulted in higher mean amounts of eicosanoids compared to buffer (30–40% increase, $P < 0.1$ to < 0.01). Perhaps this smaller effect of water on the gastric mucosa, compared to the colonic mucosa, may reflect a smaller osmotic perturbation of the gastric mucosal cell membranes. The luminal surface of the gastric mucosa is designed to withstand the osmotic effect of intraluminal water, whereas normally the colon is exposed only to iso-osmotic solutions.

In rats, ethanol (40–100%) intraluminally caused a concentration-dependent increase in LTC₄ release with no change in 6-keto-PGF_{1α} (Peskar *et al.*, 1986; Boughton-Smith & Whittle 1988a). By contrast, we found that 100% ethanol increased the amounts of PGE and 6-keto-PGF_{1α}, but had no effect on TXB₂, and actually reduced the amount of LTC₄/D₄. At the lower concentrations of 5–40%, ethanol

concentration-dependently increased the amounts of all the eicosanoids from both types of human mucosa/submucosa. However, in the *ex vivo* rat experiments (Peskar *et al.*, 1986) and the studies using the whole stomach rather than just the mucosa (Boughton-Smith & Whittle 1988a), the ethanol reached the tissue mainly via the epithelial surface. Furthermore, the mucus adhering to intact mucosa presumably diluted the ethanol before it reached the epithelial cells. By contrast, in our experiments the ethanol penetrated directly into the mucosal pieces and may therefore have reached higher concentrations than in the rat studies. This, rather than a species difference, may explain why we obtained effects at lower ethanol concentrations, and it may be more appropriate to compare our findings at lower ethanol concentrations with those at higher concentrations in the rat studies.

Increased amounts of eicosanoids due to the ethanol may have resulted from perturbation of the cell membranes. The maximum response in our experiments varied with both the ethanol concentration and the individual substances (20% ethanol for maximum LTC₄/D₄ accumulation, 40% for maximum TXB₂ and 6-keto-PGF_{1α}, and 80% for PGE in both the gastric and colonic tissues). With the gastric mucosa, 80–100% ethanol increased both the PGE and 6-keto-PGF_{1α} accumulation, but with 100% ethanol the mean amount of TXB₂ was unchanged, and LTC₄/D₄ production was inhibited. With the colonic mucosa, 100% ethanol increased PGE accumulation but considerably decreased the amounts of TXB₂, 6-keto-PGF_{1α} and LTC₄/D₄. The LTC₄/D₄ yield seems to be the most sensitive to inhibition by a high ethanol concentration. This might be because lipoxygenase is a cytosolic enzyme, and therefore more accessible to the ethanol, whereas cyclo-oxygenase is membrane-bound.

We cannot explain the differences between the two types of human mucosa/submucosa, or between different eicosanoids, in the effects of the higher concentrations of ethanol, or why increased prostanoid formation can occur with concentrations of ethanol that might be expected to denature the enzymes involved. Perhaps the 5 min incubation with the alcohol, and its dilution on diffusion through the tissue, did not allow denaturation of the cyclo-oxygenase, particularly at the centre of mucosal pieces.

At concentrations reaching the human stomach on drinking strong alcoholic beverages, gastric mucosal leukotriene synthesis might increase. If leukotriene formation contributes to gastric mucosal damage by ethanol, its effects may be antagonised by the increased amounts of prostaglandins formed, and increased by the greater thromboxane production. It would be of interest to see whether selective 5-lipoxygenase inhibitors reduce gastric mucosal damage by ethanol in man.

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