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# Cytoprotective effects of disodium cromoglycate on rat stomach mucosa

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1 The cytoprotective effects of the anti-asthmatic drug, disodium cromoglycate (DSCG), on gastric mucosal necrosis induced by ethanol in rats were studied. Subcutaneous, but not oral, DSCG prevented the formation of gastric lesions and this effect was dose-dependent between 1.25 and 40 mg kg$^{-1}$, with an $ED_{50}$ value of 6.8 mg kg$^{-1}$. Maximal cytoprotection occurred 15–30 min after DSCG treatment.

2 Histological examination revealed that DSCG effectively protected the gastric mucosa against ethanol-induced vascular congestion, haemorrhage, epithelial desquamation and mucosal oedema.

3 Enhanced production of endogenous prostaglandins, which are known cytoprotective compounds, could not explain the mucosal protection. At a dose of 40 mg kg$^{-1}$, DSCG did not change prostaglandin $E_2$ or 6-keto-prostaglandin $F_{1\alpha}$ concentrations in gastric mucosal tissue, although its cytoprotective activity was partially inhibited by prior treatment of the animals with indomethacin.

## Introduction

Disodium cromoglycate (DSCG) is widely used as a prophylactic agent in the treatment of bronchial asthma, rhinitis and conjunctivitis. Although DSCG was introduced as an anti-asthmatic drug nearly twenty years ago, its precise mode of action still remains an enigma (Pearce, 1985). Classically, the drug is believed to stabilize the cell membrane of connective tissue mast cells, thus preventing the release of allergic mediators such as histamine or leukotrienes (Schwender, 1983; Tasaka, 1985). Furthermore, DSCG is capable of blocking irritant receptors in the airways; indeed, evidence is growing that its effects on mast cell stabilization are irrelevant to its therapeutic action (Biggs & Goel, 1985).

In the present paper we describe a novel pharmacological action of DSCG. Evidence is presented that DSCG protects rats against severe destruction of their gastric epithelium produced by orally administered ethanol.

## Methods

Male Wistar rats (200–210 g) were used. The animals were placed in individual cages and fasted for 24 h. Water was withheld for 16 h before the experiments.

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### *Gastric mucosal lesion induction*

DSCG or vehicle was administered subcutaneously or orally by gavage to rats at various times before an oral load of 1 ml of absolute ethanol. One hour after ethanol treatment, animals were anaesthetized with ether. Their stomachs were ligated at the cardic and pyloric sphincters, distended by injection into the lumen of 5 ml of half-strength Karnovsky's fixative (Karnovsky, 1965), excised and immersed in the fixative for at least 1 h. After fixation, the stomach was opened along the lesser curvature and the total length (mm) of the linear haemorrhagic lesions measured for each stomach. Results are expressed as mean length of gastric lesion for each group of rats or as percentage inhibition of lesion formation compared with vehicle-treated animals. For light microscopy, samples from the gastric corpus measuring 2 × 4 mm were excised from the region located 2–3 mm below the limiting ridge which separate the forestomach oxyntic gland area. Samples were removed perpendicularly to the long axis of the stomach, fixed for an additional 2 h and routinely embedded in epon. Tissue sections (2 μm) were stained with toluidine blue and examined by an unbiased observer. In some experiments, the effect of DSCG was determined 1 h after oral administration of indomethacin (10 mg kg$^{-1}$) (Ligumsky *et al.*, 1982) to inhibit gastric prostaglandin synthesis.

*Prostanoid determination*

Rats were treated subcutaneously with DSCG (40 mg kg$^{-1}$) or solvent. Thirty minutes later, the animals were killed. Their stomach was immediately excised, opened along the lesser curvature and washed with ice cold saline containing 10 $\mu$g ml$^{-1}$ indomethacin. After blotting, the gastric corpus mucosa was carefully scraped off, weighed and placed into 1 ml of ice-cold methanol. After addition of 1 ml of 0.1 M sodium acetate (pH 4.2), the tissue was homogenized. The homogenate was centrifuged (12,000 *g*, 10 min) and the supernatant extracted twice with 6 ml of diethylether. The ether phases were combined, backwashed with 1 ml water and evaporated to dryness. The residue was dissolved in 10% ethanol and aliquots were subjected to radioimmunoassay to determine prostaglandin $E_2$ ($PGE_2$) and 6-keto-$PGF_{1\alpha}$ concentrations (Amersham).

*Statistics*

All data are presented as mean ± s.e.mean. Comparisons between groups were performed by use of Student's *t* test for unpaired data, taking $P < 0.05$ as significant. The $ED_{50}$ values with 95% fiducial limits were computed by probit analysis (Finney, 1971).

*Drugs*

Disodium cromoglycate (DSCG) and indomethacin were obtained from Sigma. DSCG was dissolved in distilled water or 0.9% w/v NaCl solution for oral or subcutaneous administration, respectively. Indomethacin, dissolved in 0.1 N NaOH at a concentration of 30 mg ml$^{-1}$, was diluted with distilled water to give a final concentration of 2 mg ml$^{-1}$. Both compounds were administered in a volume of 0.5 ml 100 g$^{-1}$ body weight. Absolute ethanol (p.a.) was obtained from Merck.

## Results

*Cytoprotective activity of DSCG*

Oral administration of absolute ethanol to control rats produced severe gastric mucosal damage. Macroscopically, this damage was confined mainly to the glandular part of the stomach and consisted of elongated lesions which had a mean total length of 85.0 ± 3.1 mm per rat ($n = 30$). Subcutaneous injection of DSCG 30 min before ethanol treatment dose-dependently prevented mucosal damage (Figure 1). The $ED_{50}$ value was 6.8 (5.2–8.7) mg kg$^{-1}$, and significant ($P < 0.05$) reduction of lesion formation was already observed after 2.5 mg kg$^{-1}$ DSCG. In contrast, oral treatment with DSCG (40 mg kg$^{-1}$, 30 min) did not result in significant cytoprotection: the mean length of enthanol-induced lesions was 61.0 ± 5.5 mm ($n = 6$) in rats pretreated with DSCG orally versus 67.0 ± 8.1 mm ($n = 6$) in solvent-pretreated animals. Light microscopy showed that in control rats the glandular mucosa had been subject to an extensive erosive process characterized by large areas of deep necrotic lesions, often extending to the muscularis mucosae, substantial loss of the epithelial sheet, striking vascular congestion with focal haemorrhages and subepithelial oedema (Figure 2a). By contrast, in animals injected subcutaneously with DSCG (40 mg kg$^{-1}$) 30 min before ethanol challenge, mucosal injury was either absent (Figure 2b) or restricted to slight and spotty shedding of the superficial epithelial cell layer (Figure 2c).

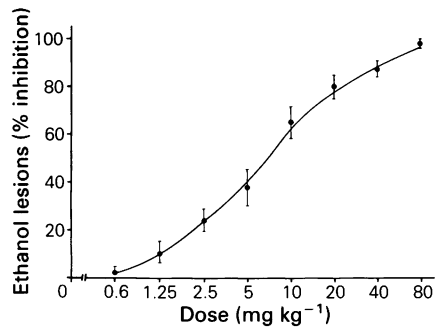


**Figure 1** Protective effect of disodium cromoglycate (DSCG) against ethanol-induced gastric necrotic lesions in rats. DSGG was administered subcutaneously 30 min before oral treatment with 1 ml of absolute ethanol. One hour later the animals were killed. Each point represents the mean of triplicate determinations from two independent experiments; vertical lines show s.e.mean. The mean lesion length per rat in control animals ($n = 30$) was 85.0 mm.

*Time course of cytoprotective activity of DSCG*

When given 15 and 30 min before ethanol gavage, a subcutaneous dose of DSCG (40 mg kg$^{-1}$) almost completely (> 90%) prevented gastric lesion formation (Figure 3). No significant inhibition of gastric necrosis was observed when the time interval between DSCG and ethanol administration was short (5 min) or prolonged (180–240 min). By interpolation, it would appear that there had been a 50% reduction of mucosal damage when DSCG was administered either 10 or 100 min before the ethanol challenge.

*Effect of indomethacin on cytoprotective activity of DSCG*

Indomethacin (10 mg $kg^{-1}$ orally, 1 h before DSCG) partially attenuated the efficacy of DSCG in preventing the formation of ethanol lesions. In the vehicle-pretreated group, DSCG (40 mg $kg^{-1}$ subcutaneously) inhibited the ethanol lesions by 91%, from $93.0 \pm 7.3$ mm to $8.2 \pm 2.2$ mm per rat ($n = 6$, $P < 0.01$), whereas in the indomethacin-pretreated group, the gastric lesions were only partially suppressed (57%) from $122 \pm 13$ mm to $52 \pm 11$ mm per rat ($n = 6$, $P < 0.02$) for vehicle- and DSCG-treated groups, respectively.

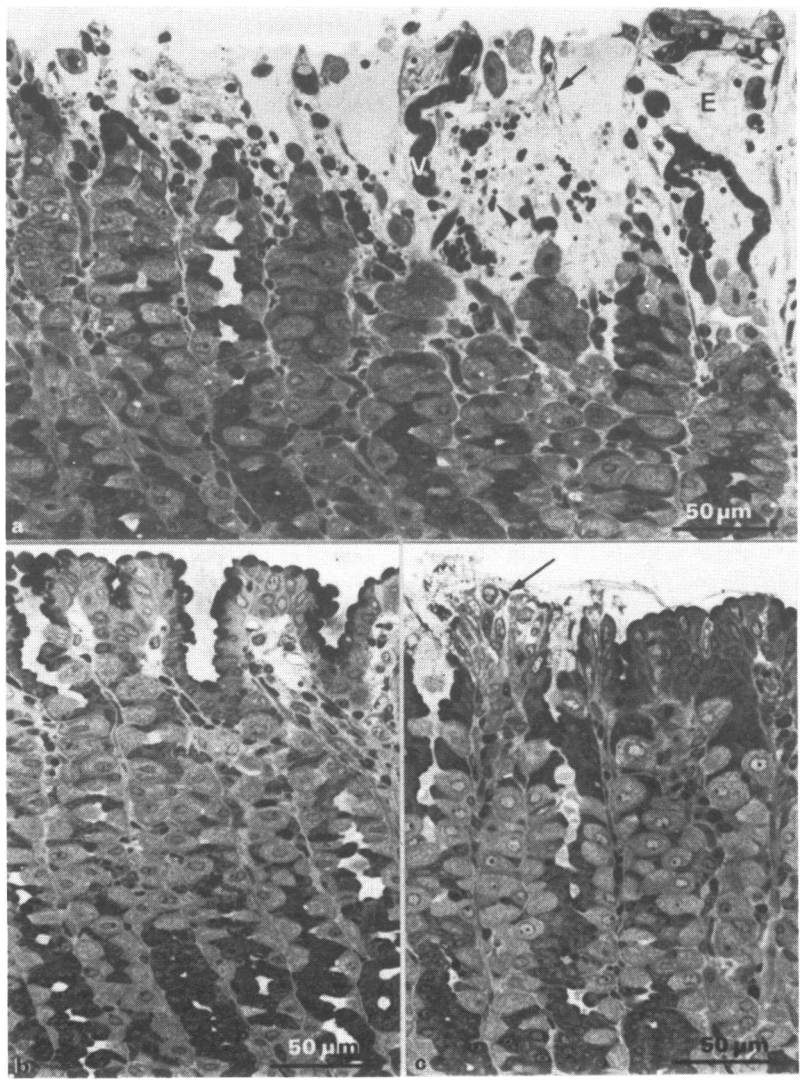


**Figure 2** Light microscopy of rat gastric mucosa. Animals were pretreated subcutaneously with either saline or disodium cromoglycate (DSCG) (40 mg $kg^{-1}$). Thirty minutes later, they were given an oral dose of 1 ml of absolute ethanol. One hour after ethanol, stomachs were fixed, glandular samples were sectioned at 2 µm and stained with toluidine blue. (a) Saline-pretreated animal: the gastric epithelium shows a severe mucosal lesion (upper right), characterized by vasocongestion (V), epithelial desquamation (arrow), focal haemorrhage (arrowhead) and subepithelial oedema (E). (b and c) DSCG-pretreated animals: the gastric epithelium is intact (b) or shows limited desquamation of the superficial epithelial cells (c, arrow).

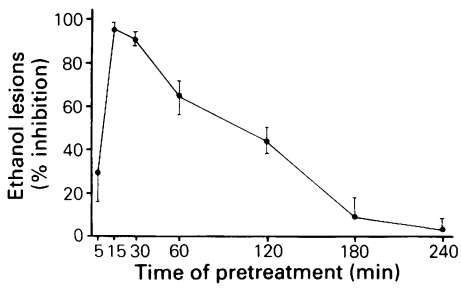


**Figure 3** Time-course of protective effects of disodium cromoglycate (DSCG) against ethanol-induced gastric lesions in rats. DSCG (40 mg kg$^{-1}$) was administered subcutaneously at different time points before oral treatment of 1 ml of absolute ethanol. Each point represents the mean of triplicate determinations from two independent experiments; vertical lines show s.e.mean.

*Effect of DSCG on mucosal prostaglandin concentrations*

The gastric mucosal content of prostaglandin $E_2$ and 6-keto-prostaglandin $F_{1\alpha}$ 30 min after a subcutaneous dose of solvent or DSCG (40 mg kg$^{-1}$) were not significantly different: for prostaglandin $E_2$, values (ng prostaglandin mg$^{-1}$ tissue) were $142 \pm 33$ in control ($n = 6$) and $179 \pm 30$ in DSCG-treated animals ($n = 6$); for 6-keto-prostaglandin $F_{1\alpha}$, values (μg prostaglandin mg$^{-1}$) were $1.04 \pm 0.06$ and $1.02 \pm 0.17$ for solvent- and DSCG-treated rats, respectively.

## Discussion

The present study demonstrates that the anti-asthmatic compound DSCG exerts cytoprotective effects in rats. The drug prevents macroscopic damage of the rat gastric mucosa produced by exposure to absolute ethanol. This effect was crucially dependent on both the route of administration and time of pretreatement: subcutaneous DSCG administered 15–30 min before ethanol challenge completely suppressed lesion formation. More prolonged (> 180 min) pretreatment periods or oral administration rendered DSCG inactive as a cytoprotective agent, in agreement with the rapid elimination of the drug from the body and its poor absorption from the gastrointestinal tract (Walker *et al.*, 1972). Histological examination indicated that DSCG protected the mucosa against damage, although in one animal out of four, the drug was unable to prevent slight desquamation of the superficial epithelial cell layer. As such, cytoprotection by DSCG resulted in a histological pattern almost identical to that found for protanoids (Lacy & Ito, 1982; Schmidt *et al.*, 1985). In spite of this similarity, however, it proved difficult to obtain clear evidence for a role of endogenous prostaglandins in the process of cytoprotection by DSCG. Indeed, although DSCG had no effect on gastric mucosal prostaglandin $E_2$ or 6-keto-prostaglandin $F_{1\alpha}$ concentrations, this drug showed reduced cytoprotective activity when endogenous prostaglandin synthesis was inhibited by pretreatment with indomethacin.

How does the cytoprotective activity of DSCG relate to its known pharmacological effects? The mast cell stabilizing action of DSCG offers no convincing explanation for its protective properties. The role of mast cells or their released mediators in the process of ethanol-induced mucosal injury is indeed questionable: specific *in vivo* antagonism of histamine at its $H_1$- or $H_2$-histamine receptor sites or of 5-hydroxytryptamine at its 5-$HT_2$-receptor, respectively, by astemizole (Van Wauwe, unpublished data), cimetidine (Robert *et al.*, 1979) or ketanserin (Van Wauwe, unpublished data) did not lead to inhibition of ethanol-induced epithelial damage.

The ability of DSCG to counteract certain platelet-activating factor (Paf)-induced responses (Basran *et al.*, 1983; Morley *et al.*, 1985) offers a more plausible explanation for its cytoprotection. Particularly meaningful in this context is the recent finding that intravenous Paf administration to rats resulted in the formation of extensive haemorrhagic erosions in the gastric mucosa (Rosam *et al.*, 1986). Unfortunately, the effect of DSCG on Paf-induced gastric injury was not investigated and, as yet, no study has been published implicating Paf as a causative agent of ethanol-induced mucosal destruction. As such, it is certainly premature to conclude that DSCG exerts its cytoprotective effects through its 'antagonistic' effect on Paf.

What could be the connection between the cytoprotective activity of DSCG and its known clinical effects? Clearly, a cytoprotective mechanism may explain the significant improvement by DSCG in the treatment of diffuse varioliform gastritis, a form of gastric mucosal inflammation (André *et al.*, 1982). However, the relation between our animal data and the anti-asthmatic activity of DSCG is not so obvious.

In a recent article, Barnes (1986) hypothesized that the bronchial hyper-responsiveness, which is a characteristic feature of asthma (Boushey *et al.*, 1980), results from eosinophil-dependent damage and destruction of the airway epithelium.

On the basis of the present data, we suggest that, analogous to its effects on gastric epithelium, DSCG could prevent the breakdown of the airway epithelium and/or accelerate its repair once damage has occurred. At present, such a suggestion remains to be experimentally proven. Although morphological abnormalities

of airway epithelium tight junctions have been demonstrated in asthma (Dunnill, 1960; Cutz *et al.*, 1978; Laitinen *et al.*, 1985), to our knowledge no studies on the effects of DSCG on airway epithelial integrity have been carried out. If substantiated, however, the proposed mechanism of action of DSCG should lead to new strategies in the pharmacological research on asthma. Our recent data indeed demonstrate that other potential anti-asthmatics such as nedocromil (Cairns *et al.*, 1985), doxanthrazole (Batchelor *et al.*, 1975) and PRD-92-Ea (El Azab & Stewart, 1977), have the same cytoprotective capability as DSCG.

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