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Ulcerogenic Mechanism of Ethanol and the Action of Sulphanilyl Fluoride on the Rat Stomach In-vivo

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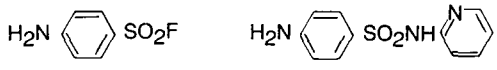
Abstract—The effects of ethanol alone and in combination with sulphanilyl fluoride on some of the antioxidant defences in the stomach of rats have been examined. These effects were correlated with lesion formation in the gastric mucosa. Oral administration of ethanol induced gastric lesions which were prevented by sulphanilyl fluoride pre-treatment. *N*-Ethylmaleimide antagonized the anti-lesion action of sulphanilyl fluoride. Ethanol administration lowered the glucose-6-phosphate dehydrogenase activity in the gastric mucosa, an effect potentiated by *N*-ethylmaleimide pre-treatment. The total superoxide dismutase activity was unaffected by the drugs used in the present study. Ethanol, however, markedly increased mucosal catalase activity which was reduced by sulphanilyl fluoride pretreatment and reversed by *N*-ethylmaleimide. It is concluded that the ulcerogenic mechanism of ethanol is mediated at least in part by the depression of the hexose monophosphate shunt and the production of active oxygen species, whereas the anti-lesion action of sulphanilyl fluoride is probably not mediated through these mechanisms.

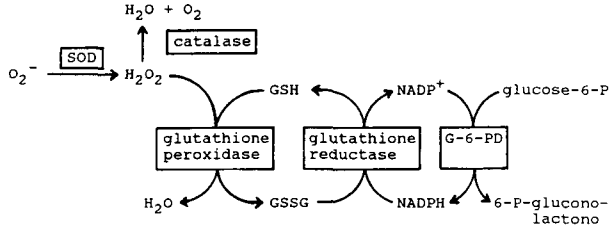
It has been shown that sulphapyridine, a metabolite of sulphasalazine, protects against ethanol ulceration (Cho et al 1987). The anti-lesion action is probably due to its sulphoxide structure. Sulphanilyl fluoride (I) which possesses a structure similar to sulphapyridine (II), could also prevent lesion formation induced by ethanol, possibly by increasing the level of protein sulphydryl (SH) which has been shown to inhibit ulceration (Szabo et al 1981).



I II

It is known that the reduced form of glutathione is cyclically regenerated by glutathione reductase with concomitant oxidation of NADPH for the hexose monophosphate shunt. One role of this shunt is to produce five-carbon units required for nucleic acid synthesis, a process of major significance in epithelial cell regeneration (Stevens et al 1953). Thus, the depression of glucose-6-phosphate dehydrogenase could relate to lesion formation in the gastrointestinal mucosa (Pfeiffer & Debro 1966; Pfeiffer & Muller 1967).

It has also been reported that oxygen-derived free radicals are involved in haemorrhagic, shock-induced gastric lesions (Itoh & Guth 1985) and ischaemic injury to the small intestine (Parks et al 1983; Granger et al 1986). The present study was undertaken to examine the effects of ethanol and/or sulphanilyl fluoride on glucose-6-phosphate dehydrogenase, and reactive oxygen scavenging enzymes (superoxide dismutase and catalase) activities in the stomach. The above phenomenon can be summarized by the following scheme.



Materials and Methods

Female Sprague-Dawley rats, 160–180 g, were housed in a temperature- and humidity-controlled room, and had free access to a standard laboratory pellet diet and tap water. Food was withheld 24 h before experiments. Each experiment was performed at least three times and results were pooled.

Sulphanilyl fluoride (Aldrich Chem. Co.) was prepared in 50% (v/v) ethanol (Fisher Scientific); *N*-ethylmaleimide (Sigma Chem. Co.) was dissolved in saline (0·9% NaCl). Sulphanilyl fluoride (0·2 or 0·8 mmol kg^{-1}) was injected s.c. 30 min before absolute ethanol administration (5 mL kg^{-1}) through an oral gastric tube. In a separate experiment, *N*-ethylmaleimide was injected s.c. 10 min after sulphanilyl fluoride treatment, i.e. 20 min before ethanol administration. The animals were killed by cervical dislocation 1 h after ethanol administration. The stomach was isolated, opened and the severity of lesions in the glandular mucosa was estimated and scored from 0–3 (0: no lesion observed, 1: petechiae, 2: haemorrhagic lesions less than 5 mm in maximal length, and 3: haemorrhagic lesions larger than 5 mm in maximal length). After the lesions were graded, the entire gastric mucosa was scraped off with a glass slide and weighed. Mucosal specimens were subsequently mixed in buffer (5 mL phosphate buffer per g tissue) and homogenized by a Polytron. The homogenates were stored at −70°C until used for determination of enzymes, total glutathione and protein.

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Determination of total glutathione and protein levels

Total glutathione (mol (mg prot.)$^{-1}$) and protein were determined by the methods of Sies & Akerboon (1984) and Bradford (1976), respectively. Bovine serum albumin was used as a standard for protein.

Measurement of enzyme activities

Glucose-6-phosphate dehydrogenase activity was measured with a Sigma Kit 345-B (Sigma Chem. Co.) based on the method of Lohr & Walker (1974) and expressed as units (mg prot.)$^{-1}$. Catalase was spectrophotometrically measured by the method of Beers & Sizer (1952) and expressed as units (mg prot.)$^{-1}$. Superoxide dismutase was assayed by the procedure of Misra & Fridovich (1972) and expressed as units (mg prot.)$^{-1}$.

Statistical analysis

The data were expressed as means ± s.e.m. and analysed by the unpaired Student's *t*-test.

Results

Effects of sulphanilyl fluoride on ethanol-induced gastric mucosal lesions

Subcutaneous injection of sulphanilyl fluoride or vehicle (50% ethanol) did not produce gastric lesions. However, oral administration of absolute ethanol produced severe gastric mucosal lesions which appeared as elongated haemorrhagic bands along the crests of glandular folds. Sulphanilyl fluoride pretreatment dose-dependently reduced the gastric lesion score (Table 1). *N*-Ethylmaleimide treatment abolished the protective action of sulphanilyl fluoride (Table 1).

Effects of sulphanilyl fluoride and/or ethanol on gastric glutathione levels

Sulphanilyl fluoride treatment alone decreased the gastric mucosal total glutathione level in a dose-related manner (Table 2). Oral administration of ethanol did not significantly affect the glutathione level (Table 2). *N*-Ethylmaleimide administration markedly reduced the gastric mucosal glutathione level (Table 3).

Effects of sulphanilyl fluoride and/or ethanol on gastric mucosal glucose-6-phosphate dehydrogenase activity

Administration of sulphanilyl fluoride did not significantly affect the glucose-6-phosphate dehydrogenase activity in the gastric mucosa. Oral ingestion of absolute ethanol significantly depressed this enzyme activity in the gastric mucosa. Sulphanilyl fluoride pretreatment did not reverse this action (Table 2). *N*-Ethylmaleimide pretreatment further reduced the gastric enzyme activity by a significant amount (Table 3).

Effects of sulphanilyl fluoride and/or ethanol on gastric mucosal superoxide dismutase and catalase activity

Neither sulphanilyl fluoride nor ethanol administration affected the total superoxide dismutase activity (Table 2).

Table 1. Effects of sulphanilyl fluoride (SF) and/or *N*-ethylmaleimide (*N*-EM) pre-treatment on ethanol (EtOH)-induced gastric lesions.

	Pretreatment (s.c.)				Treatment		No. of rats	Lesion score (0–3)
	−30 min		−20 min		0 min			
A.	50% EtOH	2 mL kg^{-1}	—	—	H_2O	5 mL kg^{-1}	9	0·0 ± 0·00
B.	50% EtOH	2 mL kg^{-1}	—	—	100% EtOH	5 mL kg^{-1}	9	3·0 ± 0·00†
C.	SF	0·2 mmol kg^{-1}	—	—	100% EtOH	5 mL kg^{-1}	10	2·5 ± 0·21
D.	SF	0·8 mmol kg^{-1}	—	—	100% EtOH	5 mL kg^{-1}	10	1·8 ± 0·024*
E.	SF	0.2 mmol kg^{-1}	Saline	2 mL kg^{-1}	100% EtOH	5 mL kg^{-1}	12	1·5 ± 0·14**
F.	SF	0·8 mmol kg^{-1}	*N*-EM	50 mg kg^{-1}	100% EtOH	5 mL kg^{-1}	12	2·9 ± 0·08§

Values indicate means ± s.e.m. †$P < 0·001$ when compared with the corresponding value in A. §$P < 0·001$ when compared with the corresponding value in E. *$P < 0·05$, **$P < 0·001$ when compared with the corresponding value in B.

Table 2. Effects of ethanol (EtOH, given p.o. at zero min) and/or sulphanilyl fluoride (SF, given 30 min before ethanol administration) on the total glutathione, glucose-6-phosphate dehydrogenase, total superoxide dismutase and catalase in the gastric glandular mucosa.

Pretreatment (s.c.)		No. of rats	Glutathione (nmol (mg prot.)$^{-1}$)	Glucose-6-phosphate dehydrogenase (units (mg prot.)$^{-1}$)	Superoxide dismutase (units (mg prot.)$^{-1}$)	Catalase (units (mg prot.)$^{-1}$)
A. Distilled H_2O	5 mL kg^{-1}, p.o.					
50% EtOH	2 mL kg^{-1}	9	7·3 ± 0·4	0·089 ± 0·007	33·5 ± 1·9	5·6 ± 0·4
SF	0·2 mmol kg^{-1}	10	6·9 ± 0·8	0·083 ± 0·004	37·0 ± 3·5	6·1 ± 0·6
SF	0·8 mmol kg^{-1}	10	5·0 ± 0·5†	0·067 ± 0·007	34·8 ± 2·6	5·7 ± 0·7
B. 100% EtOH	5 mL kg^{-1}, p.o					
50% EtOH	2 mL kg^{-1}	11	5·8 ± 1·0	0·035 ± 0·007**	38·5 ± 5·8	22·2 ± 6·0*
SF	0·2 mmol kg^{-1}	10	8·3 ± 0·5	0·046 ± 0·006**	50·8 ± 6·6	12·8 ± 1·6**
SF	0·8 mmol kg^{-1}	10	8·2 ± 1·7	0·048 ± 0·006	50·9 ± 8·4	14·9 ± 2·8**

Values indicate means ± s.e.m. *$P < 0·05$, **$P < 0·001$ when compared with the corresponding value in A. †$P < 0·05$ when compared with its own vehicle (50% EtOH)-injected control.

Table 3. Effects of ethanol (EtOH), sulphanilyl fluoride (SF) and/or *N*-ethylmaleimide (*N*-EM) treatment on the total glutathione, glucose-6-phosphate dehydrogenase, total superoxide dismutase and catalase in the gastric glandular mucosa.

Pretreatment (s.c.)		Treatment (p.o.)	No. of rats	Glutathione (nmol (mg prot.)$^{-1}$)	Glucose-6 phosphate dehydrogenase (units (mg prot.)$^{-1}$)	Superoxide dismutase (units (mg prot.)$^{-1}$)	Catalase (units (mg prot.)$^{-1}$)
−30 min	−20 min						
A. SF 0·8 mmol kg^{-1}	Saline 2 mL kg^{-1}	100% EtOH 5 mL kg^{-1}	12	12·55 ±1·19	0·041 ±0·003	57·8 ±4·5	11·1 ±1·3
B. SF 0·8 mmol kg^{-1}	*N*-EM 50 mg kg^{-1}	100% EtOH 5 mL kg^{-1}	12	7·52† ±0·75	0·017†† ±0·002	52·8 ±6·0	22·0†† ±2·4

Values indicate means ± s.e.m. †$P < 0·01$, ††$P < 0·001$ when compared with the corresponding value in A.

N-Ethylmaleimide given together with sulphanilyl fluoride and ethanol also did not change the gastric activity of this enzyme (Table 3).

Sulphanilyl fluoride injection did not affect the enzyme activity which was, however, markedly elevated after oral administration of ethanol (Table 2).

Although the level of catalase was relatively low in the sulphanilyl fluoride-injected groups, it was not at a significant level. The enzyme activity remained at a significantly high level in the gastric mucosa when compared with its respective control not subjected to oral administration of ethanol (Table 2). *N*-Ethylmaleimide injection together with sulphanilyl fluoride significantly increased the gastric mucosal catalase activity when compared with the rats treated with sulphanilyl fluoride alone (Table 3).

Discussion

The results suggest that the antagonistic action of *N*-ethylmaleimide on the protective effect of sulphanilyl fluoride is mediated by an action unrelated to its sulphydryl alkylating action and the anti-lesion action of sulphanilyl fluoride is not controlled by increasing sulphydryl level in the gastric mucosa.

Although the total glutathione level appears not to be involved in ethanol ulceration, it is noted that damage to structural proteins and enzymes induced by free radicals is caused partly by the oxidation of essential sulphydryl groups which scavenge potentially harmful free radicals. The reduced form of glutathione is cyclically regenerated by glutathione reductase and it concomitantly oxidizes NADPH for the hexose monophosphate shunt. If the above action is stopped, the hexose monophosphate shunt is also affected. A depression of activity of this major carbohydrate metabolic shunt has been observed in experiments following the imposition of dietary, pharmacological, psychological, or surgical stress (Lee et al 1962; Pfeiffer & Debro 1966; Pfeiffer & Muller 1967). The glucose-6-phosphate dehydrogenase activity is very responsive to environmental stress. The present study demonstrated such a reaction in which oral administration of ethanol markedly decreased gastric mucosal glucose-6-phosphate dehydrogenase. The importance of the hexose monophosphate shunt in the gastrointestinal tract has been suggested. It partially fulfills the large energy demand of the mucosa and produces five-carbon units required for nucleic acid synthesis, a process required for epithelial regeneration (Stevens et al 1953). A partial inhibition of this shunt, induced by exogenous glucocorticoids, may be associated with the observed ulcerogenesis following chronic administration of steroids (Hall & Captain 1953; McEwen 1957). The present study also demonstrated such an association, in which the glucose-6-phosphate dehydrogenase was depressed in the stomach and the lesions were found in the same organ. Similar results were found with *N*-ethylmaleimide, when the enzyme was further reduced (Table 3) and the protective effect of sulphanilyl fluoride was abolished (Table 1). However, it is still not clear how sulphanilyl fluoride protected against ethanol ulceration but had no effect on the activity of glucose-6 phosphate dehydrogenase.

Available data also suggest that oxygen free radicals appear to be a fundamental factor causing tissue injury during the pathogenesis of various disorders of the digestive system. Superoxide dismutase, an $O_2\cdot$ scavenging enzyme, has been shown to be a cellular protective enzyme in bowel ischaemia and against aspirin- and ethanol-induced gastric lesion formation (Dalsing et al 1983; Parks et al 1983; Mozsik et al 1984; Czegledi et al 1986). The enzyme was found to be activated after aspirin administration and was postulated to be one of the defense mechanisms against aspirin-induced lesions in the rat stomach (Czegledi et al 1986). The present study did not show any increased activity of this enzyme in the gastric mucosa after ethanol administration, although Mozsik et al (1989) reported an increase in this enzyme activity in this organ 15–60 min after ethanol administration. As the present data showed that sulphanilyl fluoride pretreatment also did not affect superoxide dismutase activity, it can be suggested that the anti-lesion action of sulphanilyl fluoride may be unrelated to superoxide production.

It was observed that ethanol markedly increased the catalase activity in the gastric mucosa, the site where lesions are mostly found after oral administration of ethanol. Perhaps catalase is the mucosal enzyme which plays an important role in detoxifying any damaging action produced by ethanol. Thus, hydrogen peroxide may be the damaging species towards biomolecules and removal of this by catalase would be a self-defense mechanism of the stomach to prevent further development of lesion formation (Lunec et al 1987). Sulphanilyl fluoride pretreatment perhaps reduced H_2O_2 production and therefore decreased lesion formation and catalase level in the stomach. *N*-Ethylmaleimide reversed the protective action of sulphanilyl fluoride and increased the lesion formation and enzyme activity. These results indicate that the severity of lesions correlates with the catalase

activity which is increased secondary to the production of H_2O_2 in the stomach.

It was noted that sulphanilyl fluoride, given alone in the doses used in this study, did not produce any observable gastric lesions and also did not affect the oxygen scavenging enzymes in the stomach. Furthermore, the drug was given s.c. instead of p.o. It is likely that its protective effect is not the result of adaptive cytoprotection.

The findings of depression of glucose-6-phosphate dehydrogenase and activation of catalase activity support the notion that both the hexose phosphate shunt and hydrogen peroxide production could play a role in ethanol ulceration. However, it is still not clear exactly how sulphanilyl fluoride prevented ethanol ulceration, although its anti-lesion action was reversed by a sulphydryl alkylator. Further, it must be borne in mind that ethanol exerts mucosal damage through other mechanisms, including prostaglandin changes, cellular hydration and blood flow changes (Lo et al 1988; Cho et al 1989) and sulphanilyl fluoride could act on these systems to protect against lesion formation.

References

Beers, R. F., Sizer, I. W. (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195: 133–140

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254

Cho, C. H., Pang, S. F., Chen, B. W., Pfeiffer, C. J. (1989) Modulating action of melatonin on serotonin-induced aggravation of ethanol ulceration and changes of mucosal blood flow in rat stomachs. J. Pineal Res. 6: 89–97

Cho, C. H., Ogle, C. W., Sevilla, E. L. (1987) The protective effects of sulphasalazine against ethanol-induced gastric damage in rats. Br. J. Pharmacol. 92: 31–37

Czegledi, B., Javor, T., Nagy, L., Patty, F., Tigyi, A., Tarnok, F., Zsoldos, T. (1986) The interrelationships between the lipid peroxidation, superoxide dismutase activity, development of gastric H^+ secretion and gastric mucosal damage produced by intragastric administration of aspirin in pylorus-ligated rats. Int. J. Tiss. Reac. 8: 23–30

Dalsing, M. C., Grosfeld, J. C., Shiffler, M. A., Vane, D. W., Hull, M., Baehner, R. L., Weber, T. R. (1983) Superoxide dismutase: a cellular protective enzyme in bowel ischemia. J. Surg. Res. 34: 589–596

Granger, D. N., McCord, J. M., Parks, D. A., Hollwarth, M. E. (1986) Xanthine oxidase inhibitors attenuate ischemia-induced vascular permeability arranges in the cat intestine. Gastroenterology 90: 80–84

Hall, B. D., Captain, M. C. (1953) Gastric ulcer associated with cortisone therapy. Ibid. 25: 80–83

Itoh, M., Guth, P. H. (1985) Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. Ibid. 88: 1162–1167

Lee, M., Debro, J. R., Lucia, S. D. (1962) Effects of thyroxine and the pattern of food intake on the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Arch. Biochem. Biophys. 98: 49–55

Lo, S. K., Leung, F. M., Guth, P. H. (1988) Protection against absolute-ethanol-induced gastric antral and corpus mucosal injury. A gross and histologic study. Dig. Dis. Sci. 33: 1403–1408

Lohr, G. W., Walker, H. D. (1974) Glucose-6-phosphate dehydrogenase. In: Bergmeyer, H. U. (ed.) Methods of Enzymatic Analysis. Academic Press, New York, pp 636–643

Lunec, J., Griffiths, H. R., Blake, D. R. (1987) Oxygen radicals in inflammation. ISI Atlas Sci. 1: 45–47

McEwen, C. (1957) Emergency aspects of corticosteroid therapy. Med. Clin. North Am. 41: 735–747

Misra, H. P., Fridovich, I. (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247: 3170–3175

Mozsik, Gy., Jávor, T., Zsoldos, T., Tigyi, A. (1984) The interrelationships between the development of ethanol-, HCl-, NaOH- and NaCl-induced gastric mucosal damage and the gastric mucosal superoxide dismutase activity in the rats. In: Mozsik, G., Par, A. (eds) Recent Advances in Gastrointestinal Cytoprotection. Akademiai Kiado, Budapest, pp 123–128

Mozsik, Gy., Suto, G., Vincze, A., Zsddos, T. (1989) Correlations between free radicals and membrane-dependent energy systems in ethanol-induced gastric mucosal damage in rats. In: Szabo, S., Pfeiffer, C. J. (eds) Ulcer Disease: New Aspects of Pathogenesis and Pharmacology. CRC Press, Boca, Raton pp 15–27

Parks, D. A., Bulkley, G. B., Granger, D. N. (1983) Role of oxygen-derived free radicals in digestive tract diseases. Surgery 94: 415–422

Pfeiffer, C. J., Debro, J. R. (1966) Stress and dietary influence on the direct oxidative pathway of carbohydrate metabolism in the intestine. Arch. Int. Physiol Biochem. 84: 97–105

Pfeiffer, C. J., Muller, P. J. (1967) Inhibitory influence of glucocorticoids on intestinal mucosal NADP-dependent enzymes in intact and hypophysectomized-adrenalectomized rats. Arch. Int. Pharmacodyn. Ther. 166: 258–272

Sies, H., Akerboon, T. P. M. (1984) Glutathione disulfide (GSSG) efflux from cells and tissues. In: Packer, L. (ed.) Methods in Enzymology vol. 105. Academic Press, New York, pp 445–451

Stevens, C. E., Daoust, R., Leblond, C. P. (1953) Rate of synthesis of deoxyribonucleic acid and mitotic rate in liver and intestine. J. Biol. Chem. 202: 177–186

Szabo, S., Trier, J. S., Frankel, P. W. (1981) Sulphydryl compounds may mediate gastric cytoprotection. Science 214: 200–202