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Cytoprotective action of carbenoxolone sodium on ethanol-induced gastric lesions in rats and its inhibition by indomethacin

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Carbenoxolone produced a marked and dose-related inhibition of ethanol-induced gastric lesions in rats. This cytoprotective action was inhibited progressively and significantly by increasing doses of indomethacin. The evidence presented confirms previous suggestions that prostaglandin(s) are involved in the cytoprotective action of carbenoxolone.

Carbenoxolone has been shown to inhibit the metabolism of prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), to increase the release of PGE_2 and reduce the formation of thromboxane B_2 (TXB_2) in gastric biopsies (Hawkey & Langman 1983; Peskar & Weiler 1983). Concentrations of PGE_2 in human gastric juice increase after carbenoxolone treatment (Rask-Madsen et al 1983). These actions of carbenoxolone may, in part, account for its ulcer-healing effects as it is well known that PGE_2 and $PGF_{2\alpha}$, at doses without anti-secretory activity, are cytoprotective whereas TXA_2, the biologically-active precursor of TXB_2, is ulcerogenic (Whittle et al 1981; Kitagawa et al 1984). Carbenoxolone also has a cytoprotective action on ethanol-induced gastric lesions in rats, with stimulation of PGE liberation into the gastric lumen (Martin et al 1983). The present study has investigated the effect of carbenoxolone on ethanol-induced gastric lesions in rats in the presence and absence of indomethacin, a potent inhibitor of prostaglandin synthesis.

Materials and methods

Male, Wistar rats (Biorex), 170 ± 20 g, were fasted for 20 h before the test, but had free access to water up to 1 h before the experiment. Indomethacin or vehicle (5% $NaHCO_3$ in 0·9% NaCl) was injected subcutaneously 75 min before the oral administration of carbenoxolone or vehicle (distilled water) to ensure optimal inhibition of prostaglandin synthesis (Chaudhury & Robert 1980). This was followed 15 min later by oral dosing with absolute ethanol (1 ml/rat). The rats were killed by cervical dislocation 1 h after being treated with ethanol. Each stomach was then excised, distended with 10 ml of 2% formol saline and preserved in the same solution for at least 4 h. The stomachs were opened along the greater curvature and examined for lesions. The total length in mm of all lesions in each rat was recorded. These results were analysed by standard linear modelling techniques using the GLIM package (Numerical Algorithms Group, Oxford).

Carbenoxolone sodium and indomethacin were in-house samples of Biorex Laboratories.

Results

Oral administration of carbenoxolone at 10, 25, 50 and 100 mg kg^{-1} caused a pronounced, dose-related inhibition of ethanol-induced gastric lesions. The ED50 and ED84 were found to be 20 and 80 mg kg^{-1}, respectively. Indomethacin at a dose of 10 mg kg^{-1} subcutaneously caused a parallel displacement of this dose-response curve to the right (P = 0·00035), changing the ED50 of carbenoxolone to 80 mg kg^{-1} (Table 1, Fig. 1).

Table 1. Shift of dose-response curve for cytoprotective action of carbenoxolone on ethanol-induced gastric lesions in rats by indomethacin. Carbenoxolone sodium was given orally 15 min before the oral administration of ethanol. All rats were killed 1 h after receiving ethanol.

Carbenoxolone sodium (mg kg^{-1})	Indomethacin (mg kg^{-1})	n	Mean lesion length (mm)	Inhibition (%)
0	0	24	100	— (1)
10	0	16	67	32
25	0	16	57	41
50	0	16	20	79 (2)
100	0	16	12	88
0	10	16	96	— (1)
25	10	8	93	6
50	10	8	55	49 (3)
100	10	8	46	53

(1) Not significantly different, P = 0·86. Combined to estimate maximal value as 99 mm.
(2) ED50 20 mg kg^{-1} (95% limits 3–160 mg kg^{-1}).
(3) ED50 80 mg kg^{-1} (95% limits 40–200 mg kg^{-1}).
Note (2) significantly differs from (3) P = 0·00035.

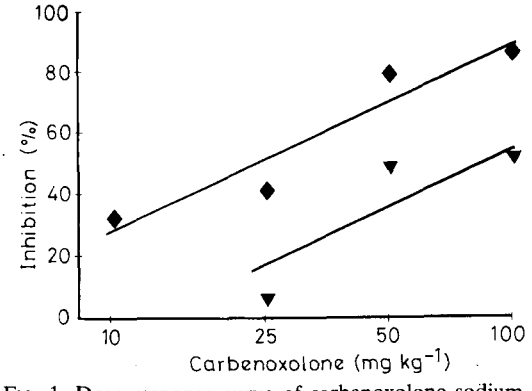

FIG. 1. Dose-response curve of carbenoxolone sodium in the presence and absence of indomethacin. Indomethacin was given as a single subcutaneous injection of 10 mg kg^{-1} 75 min before oral dosing with carbenoxolone sodium at 25, 50 and 100 mg kg^{-1}. Carbenoxolone alone (◆, n = 16 for each point). Carbenoxolone + indomethacin (▼, n = 8 for each point). Standard deviation of observations is 30 mm.

The results presented in Table 2 show that indomethacin alone at doses of 2·5, 5 or 10 mg kg^{-1} did not have a detectable effect on lesion formation. However, the cytoprotective action of carbenoxolone was reduced in a dose-related manner by indomethacin (P = 0·00033).

Table 2. Inhibition by indomethacin of the cytoprotective action of carbenoxolone sodium on ethanol-induced gastric lesions in rats. Indomethacin was injected subcutaneously 75 min before the oral administration of carbenoxolone sodium, followed by ethanol orally 15 min later. All rats were killed 1 h after receiving ethanol.

Carbenoxolone sodium (mg kg^{-1})	Indomethacin (mg kg^{-1})	n	Mean lesion length (mm)	Inhibition (%)
0	0	28	119	— (1)
0	2·5	8	112	— (1)
0	5	10	99	— (1)
0	10	10	129	— (1)
100	0	28	19	83 (2)
100	2·5	8	34	71
100	5	10	35	70 (3)
100	10	10	80	31

(1) Not significantly different, P = 0·15. Combined to estimate maximal value as 116 mm.
(2) Significantly different from maximal, $P = 10^{-6}$.
(3) Dose-dependent reduction from (2), P = 0·00033.

Discussion

The present results, on the inhibition of ethanol-induced gastric lesion formation by carbenoxolone, confirm those of Derelanko & Long (1981). These authors also reported that exogenously-administered PGE exerted a similar action to carbenoxolone.

In the present study, the action of indomethacin in reducing the cytoprotection of carbenoxolone, clearly provides the first indirect evidence that prostaglandin(s) may mediate the protective action of carbenoxolone against ethanol-induced gastric lesions.

Indomethacin has also been shown to inhibit the protective effect of arachidonic acid on ethanol-induced gastric lesions in rats by affecting prostaglandin synthesis (Hollander et al 1982; Miller & Henagan 1984). Gastric damage in rats caused by high doses of indomethacin is greatly reduced by the simultaneous administration of small quantities of carbenoxolone which also increases gastric PGE_2 levels (Martin et al 1984).

The concept of cytoprotection refers to the ability of certain agents, especially prostaglandins, in doses below those that inhibit gastric acid secretion, to protect the stomach against ulcerogenic substances (Robert et al 1979; Varró 1983). The cytoprotective action of prostaglandin(s) may be mediated via one or more mechanisms, including stimulation of mucus and/or alkaline secretion in the stomach, prevention of the disruption of the gastric mucosal barrier, modulation of gastric mucosal blood flow, normalization of DNA, RNA and protein synthesis in the gastric mucosa, regulation of ionic permeability of gastric epithelial cells to sodium and chloride ions and, possibly, enhancement of gastric mucosal cyclic(c) AMP formation (Miller 1983).

Carbenoxolone is considered to be a potent cytoprotective agent since it promotes the healing of gastric and duodenal ulcers without significant effects on gastric acid secretion (Pinder et al 1976). It increases mucus production and mucus gel thickness (Bickel & Kauffman 1981; Bradbury et al 1983; Green et al 1981), stimulates gastric mucosal blood flow (Johnston & McIsaac 1982), normalizes DNA and protein synthesis rates in gastric mucosa (Mitznegg et al 1979; Van Huis & Kramer 1981), strengthens gastric mucosal resistance to hydrogen ion back-diffusion (Birnbaum & Karmeli 1975), reduces peptic activity (Roberts et al 1983) and elevates cAMP levels in the gastric mucosa (Vapaatalo et al 1978). The remarkable similarities in the mode of action of carbenoxolone and prostaglandin(s) are highly suggestive that the cytoprotective effect of carbenoxolone might be mediated in part via the action of prostaglandin(s).

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REFERENCES

Bickel, M., Kauffman, Jr, G. L. (1981) Gastroenterol. 80: 770–775

Birnbaum, D., Karmeli, F. (1975) in: Avery Jones, F., Parke, D. V. (eds) Fourth Symposium on Carbenoxolone. Butterworths, London, pp 153–159

Bradbury, J. E., Thomas, J. M., Williams, D. G., Misiewecz, J. J. (1983) Abstracts, 58th S.R.S. meeting, Aberdeen, Paper No. 16

Chaudhury, T. K., Robert, A. (1980) Dig. Dis. Sci. New Series 25: 830–836

Derelanko, M. J., Long, J. F. (1981) Proc. Soc. Exp. Biol. Med. 166: 394–397

Green, A. P., Lander, J. E., Turner, D. H. (1981) J. Pharm. Pharmacol. 33: 348–352

Hawkey, C. J., Langman, M. J. S. (1983) Gut 24: A973

Hollander, D., Tarnawski, A., Ivey, K. J., DeZeery, A., Zipser, R. D., McKenzie, Jr, W. N., McFarland, W. D. (1982) J. Lab. Clin. Med. 100: 296–308

Johnston, B. J., McIsaac, R. L. (1982) Br. J. Pharmacol. 77: 425P

Kitagawa, H., Kurahashi, K., Fujiwara, M. (1984) Jap. J. Pharmacol. 35: 478–480

Martin, A., Gurrieri, G., Sturniolo, G. C. Naccarato, R. (1983) Advances in Prostaglandin, Thromboxane and Leukotriene Research. 12: 409–410

Martin, A., Zaramella, N., Sturniolo, G. C., Gurrieri, G., Naccarato, R. (1984) Br. Soc. Gastro. Salford Meeting, April, 1984. Abstract F47

Miller, T. A. (1983) Am. J. Physiol. 245: G601–G623

Miller, T. A., Henagan, J. M. (1984) Dig. Dis. Sci. 29: 141–149

Mitznegg, P., Domschke, S., Domschke, W. (1979) Abstracts, Gastroenterol. 76: 1204

Peskar, B. M., Weiler, H. (1983) Gut 24: A480
Pinder, R. M., Brogden, R. N., Sawyer, P. R., Speight, T. M., Spencer, R., Avery, G. S. (1976) Drugs 11: 245–307
Rask-Madsen, J., Bukhave, K., Madsen, P. E. R., Bekker, C. (1983) Eur. J. Clin. Invest. 13: 351–356
Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. J. (1979) Gastroenterol. 77: 433–443
Roberts, N. B., Walker, V., Etherington, D. J., Baron, J. H., McConnell, R. B., Taylor, W. H. (1983) Acta Gastro. Ent. Belg. 46: 448–458
Van Huis, G. A., Kramer, M. F. (1981) Gut 22: 782–787
Vapaatalo, H., Linden, I.-B., Metsa-Ketela, T., Kangasaho, M., Laustiola, K. (1978) Experientia 34: 384–385
Varró, V. (1983) Acta Physiol. Hungarica 61: 13–22
Whittle, B. J. R., Kauffman, G. L., Moncada, S. (1981) Nature 292: 472–474

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The effect of bezafibrate on hyperlipidaemia in experimental nephrotic syndrome in rats

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The effects of bezafibrate on hyperlipidaemia in experimental nephrotic syndrome in rats has been investigated. The treated group received bezafibrate 10 mg kg^{-1} p.o. daily. No significant differences in total serum cholesterol occurred, but a significant reduction in serum triglyceride ($P < 0.005$) and elevation in HDL cholesterol ($P < 0.005$) occurred. These findings may have implications for therapeutic intervention in severe hyperlipidaemia of the nephrotic syndrome in man.

The genesis and significance of the hyperlipidaemia which occurs in the nephrotic syndrome remains obscure. The changes in lipoprotein metabolism that occur are probably secondary to the hypoalbuminaemia, as an inverse relation exists between the hypoalbuminaemia and hyperlipidaemia (Baxter 1962), and infusing albumin will reduce the hyperlipidaemia (Bogdonoff et al 1961). Although a sustained elevation of serum lipids is a risk factor in arterial disease, there are conflicting opinions regarding the prevalence of arterial disease in the nephrotic syndrome (Curry & Roberts 1977; Wass et al 1979) and the role of hyperlipidaemia in its progression.

Clofibrate, a lipid lowering agent, has been used to treat essential hyperlipidaemia. However an acute muscular syndrome (myalgia) and elevations of serum creatine phosphokinase have complicated its use in treating the hyperlipidaemia of the nephrotic syndrome (Bridgman et al 1972). The myalgia seen in such patients is related to high serum drug levels consequent upon reduced plasma protein binding and renal excretion (Bridgman et al 1972).

Bezafibrate, another lipid lowering agent with different pharmacological properties to clofibrate, has been assessed with regard to its lipid lowering effect in experimental nephrotic syndrome in rats, induced by puromycin aminonucleoside.

Methods

Fifteen female Wistar rats (6 control, 9 treated), 200–275 g received a single intraperitoneal injection of puromycin aminonucleoside 70 mg kg^{-1} (Sigma Chemical Co., St Louis, USA, Lot No 109C–4009) in 1·0 ml of sterile water to induce the nephrotic syndrome (Fiegelson et al 1957; Derr et al 1968). The rats were housed in metabolic cages with free access to standard rat feed and water for 24 h before sample collection. Nine rats received a daily oral dose of bezafibrate 10 mg kg^{-1} (Boehringer Mannheim Ltd) as a suspension in 0·5% w/v methylcellulose, commencing 24 h after the administration of puromycin aminonucleoside, for the duration of the study.

Blood samples and 24 h urine samples were obtained on days 0, 7, 14 and 21. Total serum cholesterol was determined enzymatically (Cholesterol C System, Boehringer Mannheim Ltd) (Stahler et al 1977). HDL cholesterol was determined in the supernatant of serum, using a similar method, after precipitation of other lipid fractions with phosphotungstic acid and magnesium ions (Burstein et al 1970). Serum triglycerides were determined by an enzymatic colorimetric assay (Peridochrom, Boehringer Mannheim Ltd). Serum LDL were calculated by application of the Friedewald formula (Friedewald et al 1972). Serum protein was determined by the biuret method (Weichselbaum 1946), and urinary proteins were measured colorimetrically using bromocresol green (Kachmar & Grant 1976).

All values are expressed as mean ±s.e.m. Student's *t*-test was used for unpaired data.

Results

The serum proteins decreased in both groups by day 7 (control 66·5 ± 2·4 to 45·3 ± 2·6 g $litre^{-1}$, $P < 0.001$, treated group 67·0 ± 3·3 to 49·0 ± 3·5 g $litre^{-1}$, $P < 0.005$) but by week 3 had returned to near normal values (control 60·9 ± 3·5 g $litre^{-1}$, treated group 58·2 ± 2·9 g $litre^{-1}$). Urinary protein excretion rose significantly by day 7 in both groups (control 8·8 ± 1·26 to 177·6 ± 25·8 mg $24\ h^{-1}$, $P < 0.001$, treated group 17·2 ± 5·4 to 160·0 ± 37·2 mg $24\ h^{-1}$, $P < 0.001$). A similar degree of

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