glutathione present in our S9 preparations, calculated on the basis of reported values[12,13], should be about 265–388 nmoles/ml.

Glutathione splits azathioprine into 6MP and 1-methyl-4-nitroimidazole (MNI) and derivate molecules[14]. Hence, one possible explanation for the observed effects of S9 and glutathione, respectively, could be the partial or complete splitting of azathioprine to its component and derivative molecules. We therefore investigated the mutagenicity of 6MP and MNI under the same conditions as used with azathioprine. MNI was not mutagenic in strain TA1535 and its mutagenicity in strain TA100 was only manifested at concentrations 50 times higher that those used for azathioprine. 6MP was mutagenic in strains TA100 and TA1535 (table 3). At concentrations below 1500 nmoles/ml, strain TA100 was less sensitive to 6MP than to azathioprine, and TA1535 was more sensitive. Addition of S9 mix resulted in a slight increase in mutagenicity in both strains tested; addition of glutathione had no such effect.

These results show that azathioprine has mutagenic properties different from those of its component molecules. Also, though the effect of S9 mix on azathioprine mutagenicity probably result from other more complex reactions, these observations are consistent with the interpretation that the effect of S9 may be due to a partial or complete nucleophilic attack on the azathioprine molecule. This splitting of azathioprine could cause the deactivation observed with TA100 and the increase in mutagenicity with TA1535.

Future studies should elucidate whether glutathione or other nucleophilic agents present in mammalian cells could also modify the mutagenicity of azathioprine in animal models.

Table 3. Effect of 6MP in strains TA100 and TA1535

Concentration of 6MP nmoles/ml	Strains TA100		TA1535	
	− S9m	+ S9m	− S9m	+ S9m
0	123 ± 14	109 ± 12	21 ± 3	15 ± 3
75	ND	ND	25 ± 6	30 ± 5
150	256 ± 24	310 ± 27	66 ± 9	96 ± 12
300	304 ± 40	388 ± 39	96 ± 23	153 ± 38
600	332 ± 36	363 ± 38	187 ± 19	304 ± 31
1500	226 ± 29	305 ± 25	224 ± 20	341 ± 27

Mean number of *his*$^+$ revertants per plate ± SE; the values given are the mean of 3 experiments; ND, not determined; S9 m, S9 mix.

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Protection of rat gastric mucosa against ethanol injury by the new synthetic prostaglandin MDL 646[1]

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Summary. Oral administration to fasted rats of absolute ethanol produces extensive necrotic lesions of gastric mucosa as well as a massive leakage of proteins and mucus glycoproteins into gastric lumen. When the new synthetic prostaglandin MDL 646, belonging to the PGE_1 series, is administered intragastrically (2 or 10 µg/kg) 30 min before ethanol administration, a significant protection of rat gastric mucosa against alcohol injury is observed.

Prostaglandins have been shown to protect both animal and human gastrointestinal mucosa against various noxious agents, such as ethanol, hydrochloric acid, boiling water and nonsteroidal antiinflammatory drugs[2-9]. This property of prostaglandins, which is independent of their acid-inhibiting activity, has been described as 'cytoprotection'[10,11]. Ethanol, because of its lipid solubility, diffuses particularly readily into the gastric mucosal cells and breaks the apical plasma membrane[12], thus giving rise to a massive leakage of proteins and mucus glycoproteins from the mucosa into the gastric lumen as well as extensive necrotic lesions of the gastric mucosa itself. In the present study we investigated the efficacy of a single intragastric administration of the new synthetic prostaglandin MDL 646 [11,15-dihydroxy-16-methyl-16-methoxy-9-oxoprost-13-en-1-oic acid, methyl ester (8*a*, 11*a*, 15R, 16R)], belonging to the PGE_1 series, in preventing EtOH-induced damage to the gastric mucosa of rat. Gastric lesions induced by ethanol were evaluated by direct examination of the mucosa under an illuminated magnifier, and leakage of mucosal proteins and mucus glycoproteins into the gastric lumen was assessed by assaying the non-dialyzable material from the gastric lumen for proteins and glucosamine, the latter as a marker for glycoproteins.

Materials and methods. Male Wistar rats (Charles River), 120–150 g, fasted for 24 h were used. Gastric lesions were induced by absolute ethanol, according to Robert's method[2]. A watery solution (2 ml/kg) of test compound MDL-646 (2 or 10 µg/kg) was given intragastrically 30 min before oral administration of absolute ethanol (1 ml/rat), whereas control animals received only the same volume of water. Animals were killed 1 or 6 h later by chloroform inhalation, their stomachs were removed after ligature of the esophagus and pylorus, rinsed externally with cold 0.9% NaCl and opened along the lesser curvature. Stomachs were then spread out and gastric lesions evaluated under an illuminated magnifier by the percentage of the mucosal area involved. The ratio, area in treated/area in control animals, was calculated.

For examination of gastric contents, stomach content was collected and gastric mucosa accurately rinsed with 5 ml of cold 0.9% NaCl. Washings and stomach contents from animals of the same group were pooled, made to 6 M with urea, and gently shaken at 37 °C for 2 h in order to bring proteins and mucus glycoproteins into watery solution. Samples were then centrifuged at 16,000 × g for 15 min and the supernatants were dialyzed against distilled water for 48 h at 4 °C, and then assayed for protein[13] and glucosamine[14] content.

Results and discussion. Oral administration of absolute ethanol to rats produced massive necrotic lesions of the gastric mucosa, as assessed histologically by direct examination of the mucosa 1 or 6 h after alcohol administration. When MDL 646 was administered intragastrically 30 min before ethanol treatment, a significant protection of the gastric mucosa against the damaging action of alcohol was observed; the injured mucosal area was reduced to less than 10% (table 1). Moreover, in all the experiments we carried out, ethanol administration produced a massive leakage of proteins and mucus glycoproteins into the rat gastric lumen, as demonstrated by the amounts of proteins and glucosamine (a marker for glycoproteins) found in the gastric contents; as in the case of gastric lesions, this leakage was markedly inhibited by MDL 646 pretreatment of rats (tables 2 and 3). But, whereas inhibition of gastric lesions reached more than 90%, proteins and glycoprotein leakage was not inhibited by MDL pretreatment at the same extent, as inhibition reached only 65% and 54% respectively. Such results agree with the report of Eric R. Lacy and Susumo Ito[16], whose study clearly showed that 16,16-dimethyl PGE_2 orally administered to rats in doses that prevent macroscopically visible hemorrhagic necrotic lesions does not prevent the destruction of gastric mucosal cells by absolute ethanol. Indeed, they have shown that in PG-treated gastric mucosa exposed to absolute ethanol, the nonnecrotic lesion areas, which appear undamaged to the naked eye, show significant damage when examined by microscopy in semithin sections. These damaged areas are distinguishable not because the epithelial cells are destroyed but because there are focal accumulations of blood due to hyperemia and hemorrhage. Extravasal blood may be in the lamina propria or submucosa, or both, and may escape into the gastric lumen also in regions that appear normal to visual inspection. This report accounts for our results, and explains why the inhibition of protein and glycoprotein leakage into the gastric lumen is lower when compared to the inhibition of gastric lesions.

Our results fit well with some additional experimental evidence[17]: MDL 646 at the dose of 3 and 5 mcg/kg also significantly antagonized gastric lesions induced in rats by indomethacin, whereas the gastric acid secretion was practically unchanged. Since protection against challenge with absolute ethanol is at present the most reliable index of gastric cytoprotection[15] and since MDL 646 protects the gastric mucosa not only from alcohol-induced injuries, as shown by the above reported results, but also from other kinds of gastric lesions[17], we conclude that the new synthetic prostaglandin MDL 646 might be of some effectiveness as a protective agent in the prevention and treatment of gastric lesions.

As far as the cytoprotective mechanism by which this prostaglandin MDL 646 would operate is concerned, we have no experimental data at present. Its effect on gastric bicarbonate secretion and mucus production, which seem to have an important role in the protection of gastric mucosa by some prostaglandins[16], will be under investigation.

Table 1. Antagonism by MDL 646 (2 µg/kg*, and 10 µg/kg** intragastrically) of gastric lesions induced by absolute ethanol (1 ml/rat, p.o.). Rats (10 animals for each group) were killed 1 or 6 h after ethanol administration

Treatment	Degree of ulceration ± SE		% inhibition	
	after 1 h	after 6 h	after 1 h	after 6 h
Ethanol	75.0 ± 3.4	64.5 ± 3.5	–	–
MDL 646* + ethanol	4.0 ± 1.5	3.0 ± 1.3	94.7	95.6
MDL 646** + ethanol	5.5 ± 1.6	–	92.2	–
MDL 646*	0	0	–	–
MDL 646**	0	–	–	–

Table 2. Antagonism by MDL 646 (2 µg/kg, intragastrically exp. No. 1, 2, 3, 5 and 10 µg/kg exp. No. 4) of gastric mucosal protein leakage into gastric lumen induced by absolute ethanol (1 ml/rat, p.o.)

Treatment	1	2	3	4	5
A: none	0.05	0.10	0.20	0.20	0.09
B: MDL 646	0.06	0.13	n.t.*	n.t.*	0.10
C: ethanol	2.11	3.64	5.30	5.30	4.40
D: MDL 646 + ethanol	0.43	1.96	2.43	1.81	0.89
	% inhibition (D versus C)				
	79.6	46.2	54.2	65.8	79.8
	mean ± SE: 65.1 ± 6.7				

Values are expressed as total µmoles of bovine albumine found in each group (5 animals). Rats were killed 1 h (exp. No. 1, 2, 3, 4) or 6 h (exp. No. 5) after ethanol administration; * n.t., not tested.

Table 3. Antagonism by MDL 646 (2 µg/kg, intragastrically exp. No. 1, 2, 3, 5 and 10 µg/kg exp. No. 4) of gastric mucosal glycoprotein leakage into gastric lumen induced by absolute ethanol (1 ml/rat, p.o.)

Treatment	1	2	3	4	5
A: none	1.79	1.63	3.62	3.62	1.16
B: MDL 646	1.79	1.42	n.t.*	n.t.*	0.94
C: ethanol	26.03	16.53	18.86	18.86	29.01
D: MDL 646 + ethanol	8.93	9.91	11.60	7.53	9.44
	% inhibition (D versus C)				
	65.7	40.0	38.5	60.1	67.5
	mean ± SE: 54.4 ± 6.3				

Values are expressed as total µmoles of glucosamine found in each group (5 animals). Rats were killed 1 h (exp. No. 1, 2, 3, 4) or 6 h (exp. No. 5) after ethanol administration; * n.t., not tested.

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