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MUCUS SYNTHESIS AND SULFHYDRYL GROUPS IN CYTOPROTECTION MEDIATED BY DEHYDROLEUCODINE, A SESQUITERPENE LACTONE

T. Guardia, J.A. Guzman,*

Departamento de Farmacología

M.J. Pestchanker, E. Guerreiro, and O.S. Giordano

Departamento de Química Orgánica, Universidad Nacional de San Luis, San Luis, Argentina

Abstract.—The aerial parts of *Artemisia douglasiana* have been used in folk medicine as a cytoprotective agent against the development of peptic ulcer. Dehydroleucodine [1], its active principle, significantly prevents the formation of gastric lesions induced by the exposure of the rats to absolute EtOH. It was found in this study that (a) pretreatment of rats with 1 (40 mg/kg, po) caused a significant increase in glycoprotein synthetic activity, approximately sevenfold as large as that of the control; and (b) pretreatment of the rats with the thiol reagent *N*-methylmaleimide (NEM) significantly diminished the cytoprotection provided by 1. However, the protective effect of 1 was not totally abolished by pretreatment with a combination of indomethacin and NEM, indicating additional mechanisms are involved in the cytoprotective action of 1.

The aerial parts of *Artemisia douglasiana* Besser (Compositae) (1) have been used in folk medicine as a cytoprotective agent against the development of peptic ulcer. Dehydroleucodine [1], an active principle, was isolated, and its pharmacological properties analyzed. We have demonstrated that 1 significantly prevented the formation of gastric lesions induced by necrotizing agents (EtOH, 0.6 N HCl, 0.2 N NaOH, and 25% NaCl) and inhibited dose-dependently the formation of gastric erosions (2).

In this study, the mechanism of the cytoprotective effect of 1 (2) was investigated with regard to: (a) the rate of gastric mucus synthesis (incorporation of *N*-acetyl-D-[1-^{3}H]glucosamine into rat gas-

tric glycoproteins) and (b) the role of the gastric endogenous sulfhydryl groups.

Pretreatment of rats with 1 (40 mg/kg, po) caused a significant increase in the glycoprotein synthetic activity, approximately sevenfold as large as that of the control value (Figure 1).

As shown in Table 1, after giving 1 ml/rat of absolute EtOH, the development of gastric mucosal damage was almost totally inhibited by 1. *N*-Ethylmaleimide (NEM) potentiated the gastric hemorrhagic lesions induced by absolute EtOH. When NEM was given first (before EtOH), the protective effect of the orally administered 1 was diminished. Indomethacin decreased the gastric mucosal protection evoked by 1. After the combined pretreatment with indomethacin plus NEM, a residual protective effect of 1 could be observed.

The gastrointestinal mucosa synthesizes several prostaglandins, including PGE$_2$, PGF$_2$, PDG$_2$, and PGI$_2$ (3). Exposure of the glandular mucosa of the rat stomach to high concentrations of EtOH rapidly produces grossly evident focal hemorrhagic lesions that can be prevented or reduced in severity by pretreatment with prostaglandins (PGs) (4) and sulfhydryl drugs (5). It is well known that
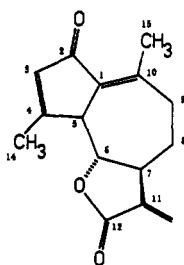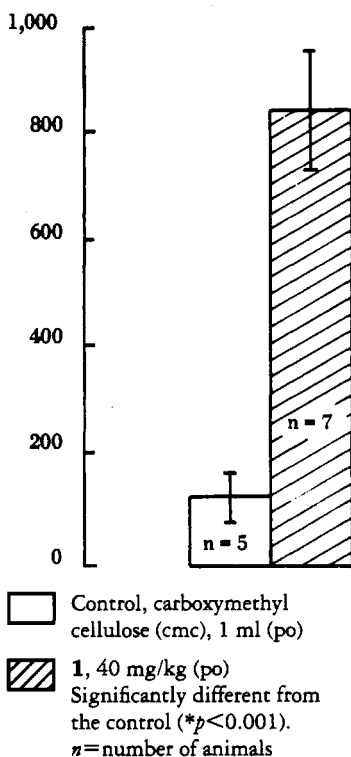

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FIGURE 1.   Effect of Dehydroleucodine [1] on the Incorporation of N-Acetyl-D-[1-³H]glucosamine into Rat Gastric Glycoproteins (in corpus).

Legend:
- □ Control, carboxymethyl cellulose (cmc), 1 ml (po)
- ▨ 1, 40 mg/kg (po) Significantly different from the control (*$p<0.001$). n=number of animals

several PGs increase the gastric mucosal blood flow (6,7) and stimulate synthesis and release of gastric mucus (8,9).

Compound **1** does not inhibit the acid secretion in the pylorus-ligated rat

(unpublished observation). It prevents formation of the gastric mucosal lesions induced by absolute EtOH and by other necrotizing agents. Indomethacin pretreatment resulted in a significant reduction of the cytoprotective action of **1** (2).

Drugs that suppress aggressive factors or intensify defensive factors have been used to treat peptic ulcer. Cimetidine is a typical example of the former group, and carbenoxolone of the latter. This compound is considered to cure peptic ulcer by stimulating mucus secretion and increasing the life span of the gastric epithelia (10). Our results suggest that **1** exerts its cytoprotective effects by a carbenoxolone-like mechanism.

Szabo (11) stressed that gastric cytoprotection might be mediated by at least two different mechanisms, one concerning PG and the other involving SH-containing compounds of the mucosa. Therefore, we investigated whether an additional SH-dependent pathway is also involved in dehydroleucodine [1] cytoprotection.

Our present results clearly show not only a significant increase in the glycoprotein synthetic activity, but also an involvement of SH-compounds of the gastric mucosa in the prevention of hemorrhagic gastric lesions induced by EtOH. The adaptative cytoprotection was de-

TABLE 1.   Effect of Pretreatment with N-Ethylmaleimide (NEM) and/or Indomethacin on Dehydroleucodine [1] Evoked Protection of Gastric Mucosa Against EtOH-Induced Damage.

Treatment prior to administration of EtOH		Extent of mucosal damage (ulcer factor)
60 min	30 min	
CMC*	CMC	4.50 ± 0.15 (10)[b]
CMC	1 (40 mg/kg po)	0.30 ± 0.16 (9)[c]
NEM (10 mg/kg sc)	CMC	5.20 ± 0.16 (9)[d]
Indomethacin (10 mg/kg sc)	CMC	4.60 ± 0.21 (6)
NEM (10 mg/kg sc)	1 (40 mg/kg po)	2.40 ± 0.29 (12)[d]
Indomethacin (10 mg/kg sc)	1 (40 mg/kg po)	1.20 ± 0.18 (7)
NEM plus indomethacin	1 (40 mg/kg po)	3.30 ± 0.30 (13)[d]

*CMC (carboxymethyl cellulose) given intragastrically served as control.
[b]Number of animals given in parentheses.
[c]$p<0.001$ in comparison to the control group (CMC-CMC).
[d]$p<0.001$ in comparison to the treatment CMC-**1**. All values were expressed as mean±SEM. Statistical analysis was carried out by unpaired Student's t-test.

creased after pretreatment with NEM. However, the cytoprotection effect of **1** was still observed, indicating that additional mechanisms could be involved in the cytoprotective action of **1**.

The involvement of at least two different pathways in the protective mechanism of **1** was demonstrated by a reduction in its cytoprotective effect after a pretreatment with indomethacin plus NEM.

EXPERIMENTAL

DETERMINATION OF RATE OF MUCUS SYNTHESIS (INCORPORATION OF N-ACETYL-D-[1-^{3}H]GLUCOSAMINE INTO RAT GASTRIC GLYCOPROTEINS).—The method employed to determine the rate of mucus synthesis in the stomach was similar to that described by Lukie and Forstner (12). Male Wistar rats (ca. 180 g) were fasted for 24 h and deprived of water for 19 h prior to the experiments. All rats were housed in wire-mesh bottom cages throughout the study to prevent coprophagy.

Dehydroleucodine [**1**] (40 mg/kg) suspended in 0.4% carboxymethyl cellulose (CMC) was administered orally to 7 rats, and 1 h later the animals were decapitated. The control rats ($n=5$) were given 0.4% CMC (1 ml). Portions of the corpus were punched out in circles, 14 mm in diameter, to make similar tissue sizes. Each tissue was preincubated at 37° for 10 min in 2 ml of Krebs medium, pH 7.4, before the addition of 1 μCi of N-acetyl-D-[1-^{3}H]glucosamine (Radiochemical Centre, Amersham). Incubation was stopped by cooling with ice, draining the medium, and washing the tissue twice with 5 ml of ice-cold saline solution. The tissue was then homogenized in 20 ml of 5 mM EDTA, pH 7.4, under cooling. Glycoproteins were precipitated overnight at 4° with 10% trichloroacetic acid–1% phosphotungstic acid. The precipitable glycoproteins were washed twice with ice in cold saline and were extracted twice with CHCl$_3$-EtOH (1:1, v/v) for defatting. The resultant glycoproteins were dried, weighed accurately, and solubilized with the addition of 0.5 N NaOH. One-half ml of the solution was neutralized with HCl and mixed with 10 ml of Insta-gel (Packard) prior to the determination of the radioactivity in a Beckman LS-100 liquid scintillation spectrometer.

SULFHYDRYL BLOCKER GROUPS.—Prior to the experiments, the rats were deprived of food for 24 h. During this period, animals were housed in individual cages to avoid coprophagy.

Gastric mucosal damage was induced by absolute EtOH (1 ml/rat). Compounds or vehicle were given either intragastrically or subcutaneously 60 or 30 min prior to the administration of EtOH as indicated in Table 1. Animals were sacrificed 60 min after application of EtOH. The stomach was removed and opened along the greater curvature. The degree of erosion in the glandular part of the stomach was assessed from a scoring system designed by Marazzi-Uberti and Turba (13) as follows: 0: no erosions; 1: 1–3 small erosions (4-mm diameter or smaller); 2: more than 3 small erosions or one large erosion; 3: one large erosion and more than 3 small erosions; 4: 3–4 large erosions; and 5: more than 3–4 large erosions and/or ulcer perforation.

The results were expressed in terms of an ulcer factor (UF) which is the sum of animals with average severity of erosions per rat for each group on the scale from 0 to 5.

All values were expressed as mean±SEM. Statistical analysis was carried out with unpaired Student's *t* test.

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