Dual Effects of N-Ethylmaleimide on Ethanol-Induced Gastric Lesions in Rats

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The effects of N-ethylmaleimide (NEM), a sulfhydryl (SH) blocker, on ethanol-induced gastric lesions were investigated in rats by varying the route of administration. Oral administration of acidified ethanol (60% ethanol in 150 mM HCl, 1 ml) produced hemorrhagic bandlike lesions in the gastric mucosa. Pretreatment of the animals with orally administered NEM (0.1–10 mg/kg) dose-dependently inhibited these lesions (the inhibition was over 80% at 1 mg/kg or greater), and the effects were partially reversed by indomethacin (5 mg/kg, subcutaneous). However, when NEM (10 mg/kg) was given subcutaneously, this agent significantly worsened the lesions. Intragastrically applied NEM produced a dose-dependent reduction of the transmucosal potential difference (PD) and the mucosal nonprotein SH levels, an increase of the volume of gastric contents, and an inhibition of gastric motility, while these parameters remained unaltered after subcutaneous administration of the agent. The microvascular permeability in the mucosa was significantly increased by both oral and subcutaneous administration of NEM (10 mg/kg) but remained unchanged in response to lower doses of orally administered (<3 mg/kg). These results suggest that NEM given orally is cytoprotective to the stomach against ethanol, probably by acting as a mild irritant and due to dilution of an irritant and inhibition of gastric motility (muscle relaxation), but when given subcutaneously it aggravates the lesions by unknown mechanisms.

KEY WORDS: ethanol; gastric lesion; N-ethylmaleimide; sulfhydryl; route of administration.
by orally administered NEM and the role of endogenous SH in gastric cytoprotection.

**MATERIALS AND METHODS**

Male Sprague-Dawley rats (230–250 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to tap water for 24 hr prior to the experiments. All studies were carried out using four to nine rats per group.

**Induction of Gastric Mucosal Lesion.** The animals were given 1 ml of acidified ethanol (60% ethanol in 150 mM HCl) orally by esophageal intubation, and they were killed 1 hr later. The stomachs were removed, inflated by injecting 10 ml of 2% formalin, immersed in 2% formalin for 10 min to fix the gastric wall, and opened along the greater curvature. The area (mm²) of each lesion was measured under a dissecting microscope with a square grid (×10), summed per stomach, and used as a lesion index. N-Ethylmaleimide (NEM) was given either subcutaneously (10 mg/kg) or orally (0.1–10 mg/kg) 30 min before ethanol treatment. In some cases, indomethacin (5 mg/kg) was given subcutaneously 30 min before administration of NEM. In all experiments, the person measuring the lesions did not know the treatments given to the animals. Some tissue samples were immersed in 10% formalin, processed for the routine microscopic observation, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E).

**Determination of Transmucosal Potential Difference.** Transmucosal PD was measured in the anesthetized rat stomachs according to the previously published method (5). Briefly, the animals were anesthetized with intraperitoneally administered urethane (1.25 g/kg), the abdomen was incised, and the stomach exposed. Both the pylorus and the lower end of esophagus were ligated, and an acute fistula, prepared by means of a polyethylene tube, was implanted in the forestomach. PD was determined using two agar bridges, one positioned in the glandular part of the stomach and the other in the abdominal cavity. The stomach was filled with 1 ml of saline (154 mM NaCl), and the PD was continuously monitored on a Hitachi recorder (model 056; Mito, Ibaraki, Japan). Approximately 30 min after basal PD had stabilized, NEM was given either subcutaneously (10 mg/kg) or intragastrically through the acute fistula implanted in the forestomach (0.3–10 mg/kg). Intragastric administration of NEM was performed after the gastric contents had been completely withdrawn. Thirty minutes later, the gastric contents were collected and both the volume and pH were measured.

**Determination of Gastric Motility.** Because recent studies suggested that inhibition of gastric motility (relaxation of stomach smooth muscle) may be associated with the phenomenon of gastric cytoprotection (6–8), the effects of NEM on motility were examined using a balloon, according to a previously published method (9). Briefly, under ether anesthesia, a balloon (containing about 0.8 ml of water), the support catheter, and another catheter for intragastric administration of drugs were placed in the glandular part of the stomach through an incision of the forestomach. The animals then were placed in Bollman cages and the support catheter was connected to a pressure transducer and polygraph device (Nihon Koden, Tokyo, Japan). Gastric motility was monitored continuously as intraluminal pressure recordings after a complete recovery from anesthesia. Quantitative analysis of gastric motility was performed by counting the number of contractions with an amplitude of 15 cm H₂O or greater and by measuring the amplitude of each contraction over a 10-min period, determining the mean of a rat for this period from these values, and by calculating the mean ± SE for each time period from five rats per group. NEM was given either intragastrically through the catheter (1–10 mg/kg) or subcutaneously (10 mg/kg) after basal motility had become well stabilized. In each test, gastric motility was measured for a total period of 2 hr.

**Determination of Mucosal Vascular Permeability.** Szabo and Trier (10) proposed the mucosal vasculature as the main target of cytoprotective drugs, while we previously reported that the enhanced vascular permeability may be responsible for aggravation of ethanol-induced gastric lesions caused by NEM and adrenalectomy (11). Therefore, we used a dye method to evaluate the effects of NEM given by different routes on the mucosal microvascular permeability (12). The animals were given NEM, either orally (0.3–10 mg/kg) or subcutaneously (10 mg/kg), and they were killed 1.5 hr later. In each case, 1 ml of Evans blue (1% w/v) was injected intravenously in the tail vein 30 min before sacrifice. Under ether anesthesia, the animals were killed by bleeding from the descending aorta, and the stomachs were removed. After collecting the gastric contents carefully by lavage with 5 ml of cold distilled water, the stomach was opened along the greater curvature, and the corpus mucosa was scraped off the lesser curvature. The mucosal vascular permeability was determined by counting the number of contractions with an amplitude of 15 cm H₂O or greater and by measuring the amplitude of each contraction over a 10-min period, determining the mean of a rat for this period from these values, and by calculating the mean ± SE for each time period from five rats per group. NEM was given either intragastrically through the catheter (1–10 mg/kg) or subcutaneously (10 mg/kg) after basal motility had become well stabilized. In each test, gastric motility was measured for a total period of 2 hr.

**Measurement of Mucosal Sulfhydryl Contents.** The amount of nonprotein SH was measured in the gastric mucosa of rats following oral (0.1–10 mg/kg) and subcutaneous (10 mg/kg) administration of NEM, according to the modified method described by Kaplowitz et al (13). Absorbance of samples was measured at 620 nm on a Hitachi spectrophotometer (model 200-100, Mito, Ibaraki, Japan). Total amount of dye trapped in the stomach for 30 min was calculated from the amount of dye recovered from the gastric contents and the mucosa and expressed as micrograms per stomach.

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