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Prevention of Ethanol-Induced Gastric Damage by the Imidazobenzodiazepines Ro 15-4513 and Ro 15-3505 in Rats

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NAJIM, R. A. AND K. H. KARIM. *Prevention of ethanol-induced gastric damage by the imidazobenzodiazepines Ro 15-4513 and Ro 15-3505 in rats.* PHARMACOL BIOCHEM BEHAV **45**(3) 597–599, 1993.—The protective effects of the imidazobenzodiazepines Ro 15-4513 and Ro 15-3505 against ethanol-induced gastric damage were investigated. Gastric lesions were induced in rats by the oral administration of 1 ml of absolute ethanol. Ro 15-4513 (2.5–10 mg/kg, IP) or Ro 15-3505 (5–20 mg/kg, IP), administered 30 min before ethanol, protected against ethanol-induced gastric damage. The protective effects of these compounds were blocked by the benzodiazepine antagonist, flumazenil (10 mg/kg, IP). These results present evidence for the involvement of the GABA-benzodiazepine receptor complex in the pathogenesis of ethanol-induced gastric damage.

Ethanol Flumazenil Gastric damage Rats Ro 15-4513 Ro 15-3505

SEVERAL recent reports have indicated that the imidazobenzodiazepines Ro 15-4513 and Ro 15-3505 antagonized the behavioral and neuropharmacological effects of ethanol in animals (1, 2, 6, 8). In addition to the behavioral and neuropharmacological effects of ethanol, several other studies have indicated that ethanol administered orally at concentrations greater than 35% (vol./vol.) caused focal areas of marked mucosal damage ranging from hyperemia, necrosis to mucosal and submucosal hemorrhages (3, 4, 9, 10).

In light of the above facts, the present investigation was undertaken to study the possible protective effects of the two imidazobenzodiazepine compounds, Ro 15-4513 and Ro 15-3505, against ethanol-induced gastric damage in rats. The reversibility of the protective effects of the two compounds by the central type benzodiazepine antagonist, flumazenil (5), was also investigated.

METHOD

Animals

Male albino Wistar rats weighing 150–200 g, obtained from the local breeding colony, were used. Animals were housed in groups of six per cage and had free access to a pellet diet and tap water. Twenty-four hours prior to the experiments, animals were transferred to a wire meshed cage and food deprived, but permitted free access to water until two hours prior to ethanol administration when water was also withheld.

Ethanol-Induced Gastric Damage

On the day of the experiment, animals received either the vehicle, Ro 15-4513 (2.5, 5 or 10 mg/kg) or Ro 15-3505 (5, 10 or 20 mg/kg) intraperitoneally 30 min before ethanol. When flumazenil was used, it was administered intraperitoneally (10 mg/kg) 45 min before ethanol administration. Absolute ethanol (1 ml) was administered orally via a stainless steel gastric tube.

One hour later, the animal was killed by a sharp blow on the head. The stomach was dissected, inflated with 1% formalin and placed in a 1% formalin bath for 5 min. The stomach was then opened along the greater curvature and examined under a dissecting microscope at 40 × magnification. The number of lesions in the glandular portion of the stomach as well as their lengths were noted and the lesion area calculated.

Drugs

Absolute ethanol (BDH) was used to induce gastric damage. Ro 15-4513 (Ethyl 8 azido-5,6 dihydro-5-methyl-6-oxo-4H imidazo [1,5-a][1,4] benzodiazepine-3-carboxylate, Ro 15-3505 (Ethyl 7-chloro-5,6-dihydro-5-methyl-6-oxo 4H imidazo [1,5-a][1,4] benzodiazepine-3-carboxylate) and flumazenil (Ro 15-1788) (Ethyl 8 fluoro-5,6-5-methyl-6-oxo 4H imidazo [1,5-a][1,4] benzodiazepine 3 carboxylate) were kindly supplied by Hoffmann La-Roche (Basel, Switzerland). The drugs were suspended in normal saline to which Tween 80 was added (2 drops of Tween 80 in 10 ml of normal saline). All drugs were freshly prepared before administration. Drug concentrations were adjusted so that each rat received no more than 2 ml/kg body weight of the drug solution. Control animals received an equivalent volume of the vehicle only.

Statistics

Statistical comparison of two groups was performed by an independent Student's *t*-test. $p<0.05$ was considered to be the minimum for statistical significance.

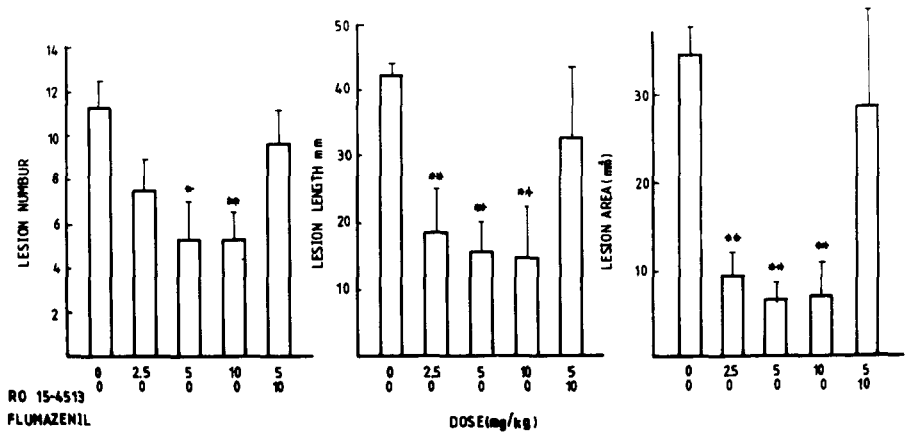

FIG. 1. Effect of vehicle (00), Ro 15-4513 or a combination of flumazenil and Ro 15-4513 on ehtanol-induced gastric damage in rats. Flumazenil was injected IP 15 min before Ro 15-4513 or the vehicle, which in turn were injected IP 30 min before the administration of ethanol (p.o., 1 ml/rat). The rats were killed 1 h after ethanol administration. Data expressed as mean ± s.e.m., n = 6. *$p < 0.01$, **$p < 0.001$.

RESULTS

Ethanol-Induced Gastric Damage

Administration of 1 ml of absolute ethanol orally to rats resulted in the production of marked mucosal damage in the glandular segment of the stomach in 100% of the rats. Lesions consisted of well defined hemorrhagic lesions, erosions and petechial hemorrhages spread throughout the glandular region.

Effect of Ro 15-4513 on Ethanol-Induced Gastric Damage

Ro 15-4513 in a dose of 2.5 mg/kg reduced the ulcer length and area significantly ($p<0.001$) (Fig. 1). When Ro 15-4513 was administered in a dose of 5 or 10 mg/kg 30 min before ethanol administration, a significant reduction in all ulcer parameters was noted as compared to the group treated with the vehicle followed by ethanol ($p<0.001$) (Fig. 1). Injection of flumazenil (10 mg/kg) 15 min before Ro 15-4513 (5 mg/kg) reversed the protective effect of the latter against ethanol-induced gastric damage (Fig. 1).

Effect of Ro 15-3505 on Ethanol-Induced Gastric Damage

Ro 15-3505 in a dose range of 5–20 mg/kg dose dependently protected against ethanol-induced gastric damage (Fig. 2). The protective effect of Ro 15-3505 (10 mg/kg) was blocked by pretreatment with the benzodiazepine antagonist, flumazenil (10 mg/kg) (Fig. 2).

DISCUSSION

Our results show that the benzodiazepine inverse agonists Ro 15-4513 and Ro 15-3505 prevented ethanol-induced gastric damage. Their protective effect was reversed by the central benzodiaz-

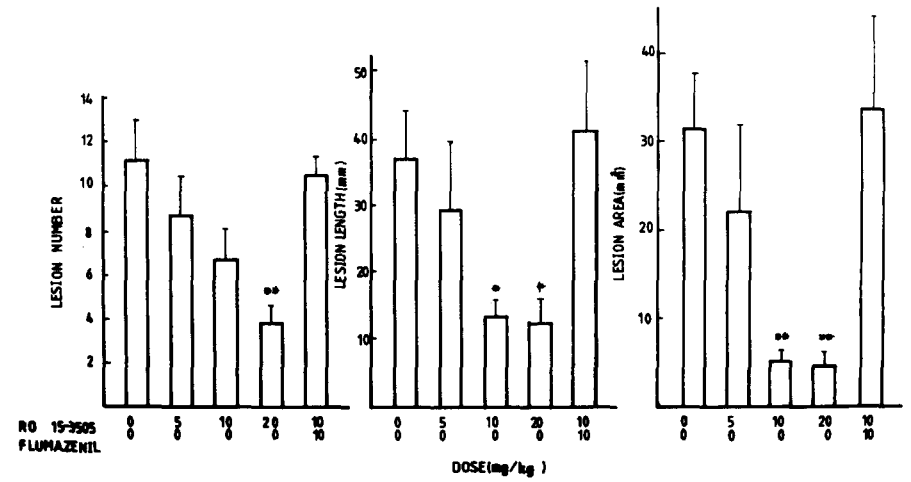

FIG. 2. Effect of vehicle (00), Ro 15-3505 or a combination of flumazenil and Ro 15-3505 on ehtanol-induced gastric damage in rats. Flumazenil was injected IP 15 min before Ro 15-3505 or the vehicle, which in turn were injected IP 30 min before the administration of ethanol (p.o., 1 ml/rat). The rats were killed 1 h after ethanol administration. Data expressed as mean ± s.e.m., n = 6. *$p < 0.01$, **$p < 0.001$.

epine antagonist, flumazenil. Flumazenil was shown not to affect ethanol-induced gastric damage when administered 45 min before ethanol in a dose range of 5–20 mg/kg (9).

Although Ro 15-4513 and Ro 15-3505 have been reported previously to block behavioral and neuropharmacological effects of ethanol (1, 2, 6), this is the first report on the reversal, by these two agents, of ethanol-induced organic damage.

Ethanol-induced gastric damage has been attributed to the release of various local vasoactive agents in the stomach leading to vascular changes which are responsible for the lesions seen (10). In fact, ethanol-induced gastric damage was grouped together with gastric damage induced by nonsteriodal antiinflammatory agents as chemically-induced gastric damage (3). However, previous published reports about the neuropharmacology of ethanol, taken together with results obtained in this study, support the view that ethanol-induced gastric damage is not totally mediated by local factors alone, but may at least in part be mediated by action of ethanol at the central GABA-benzodiazepine receptor complex. Thus ethanol has been reported to act at the GABA-benzodiazepine receptor complex (12), and to increase receptor mediated Cl^- transport mechanism (11). Ro 15-4513 and Ro 15-3505, both partial inverse agonists at the benzodiazepine receptor (1,8), in this study protected against ethanol-induced gastric damage and their protective effects were reversed by flumazenil the benzodiazepine antagonist (5).

The finding that Ro 15-3505 appeared less effective than Ro 15-4513 agrees with previous published reports that Ro 15-4513 has a more marked inverse agonistic action than that of Ro 15-3505 (2,7).

In conclusion, this study indicates the possible involvement of the GABA-benzodiazepine receptor complex in ethanol-induced gastric damage. This might open a new perspective in the understanding of the pathophysiology of gastric damage in general.

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