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Diazoxide, a K_{ATP} Channel Opener, Prevented Ethanol-Induced Gastric Ulceration in Rats

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ABSTRACT

Ethanol-induced acute gastric ulceration (EIGU) is widely studied. ATP dependent potassium channel (K_{ATP}) modulators are thought to interfere with some physiologic functions of the stomach. We have studied the effects of different doses of K_{ATP} modulators (diazoxide as agonist and glibenclamide as antagonist) on EIGU in rats. Gastric lesions were quantified. Fasting blood glucose (FBS) levels were measured enzymatically. EIGU was prevented by diazoxide and aggravated by glibenclamide. Diazoxide increased and glibenclamide decreased FBS respectively. The present study shows that K_{ATP} modulators are involved in the production of EIGU with a mechanism which remains to be elucidated.

Keywords: *ethanol, potassium channel modulators, rats, stomach ulceration*

Ethanol-induced gastric ulceration (EIGU) in rats is considered to be a reliable tool to study the pathogenesis of acute gastric mucosal ulceration.

According to Yonei and Guth [1] the gastric submucosal microvascular disturbance resulting in local ischemia is an important early reaction following the use of ethanol. The endogenous mediators for the early vascular damage of the gastric mucosa include: nitric oxide [2], leukotrienes [1, 3], histamine [4], adenosine [5], TNFα [6] and endothelins [7]. Szabo and Co-workers suggested that the release of endothelins plays a role not only in the pathogenesis of EIGU but also in the process of ulcer healing. For a long time the vagus nerve was believed to play a part in the physiologic functions of the stomach as well as some pathologic conditions. Moreover, it had been known that the CNS control of the gastric motility, acid secretion and to some extent, blood flow, was via the vagus nerve. The above factors were suggested to be of importance in the production of EIGU. Upon stimulation, primary afferent parasympathetic nerve terminals, in the gastric mucosa, release CGRP resulting in a protective hyperemia. The CGRP-induced vasodilatation appears to be mediated by K_{ATP} channel modulation [8].

Potassium channels represent the largest and most diverse family of ion channels in the body. ATP-dependent K^+ channels (K_{ATP}) are a class of ligand gated proteins. They have been postulated to be involved in a variety of physiologic functions of the stomach such as gastric blood flow regulation, acid secretion and stomach contractility [9].

To evaluate the possible part played by modulators of ATP-dependent K^+ channels, we have studied the effects of diazoxide, a potent agonist and glibenclamide, a potent antagonist of K_{ATP} channels, on EIGU in rats.

The effects of enalapril (a vasodilator with mechanism of action independent of K_{ATP} channels) were also studied in the ethanol-ulcerations. To elucidate the possible involvement of blood glucose level in these conditions, the fasting blood sugar (FBS) of K_{ATP} modulator treated animals were also measured.

MATERIALS AND METHODS

Male rats of Sprague-Dawley strain, weighing 170-200 g were used. They were fasted for 24 hours but had access to water ad libitum. Groups of ten rats were used. Volume of all drugs was 1 ml/100 g of animal's body weight. The first group received 1 ml of 0.9% normal saline. Groups 2, 3 and 4 received 5, 15 and 45 mg/kg of diazoxide (in a preliminary experiment we did not observe any effect on EIGU by using lower doses of diazoxide). Groups 5, 6 and 7 received 2, 6 and 18 mg/kg of glibenclamide. Groups 8, 9 and 10 received enalapril at the doses of 10, 20 and 40 mg/kg. The above drugs were administrated by the i.p. route. Thirty minutes later, animals in groups 1-10 received ethanol 60% at the dose of 1 ml/rat by orogastric intubation.

Rats in groups 11, 12, and 13 received the same doses of diazoxide as groups 2, 3 and 4, respectively.

Groups 14, 15 and 16 were treated with the same doses of glibenclamide as groups 5, 6 and 7. Groups 17, 18 and 19 received the same doses of enalapril as groups 8, 9 and 10. Thirty minutes later animals in groups 11-19 received 1 ml/rat of saline solution by the oral route and were considered as controls. Animals were sacrificed 120 min after the use of the last drug. Blood sample was taken for glucose measurement. This was determined enzymatically. The rat's abdomen was opened. The stomachs were removed, opened along the greater curvature and checked for the presence of ulceration. This was done by a person who was not aware of the treatment given to rats. Ulcerations were the linear necrohemorrhagic lesions present in the glandular part of the stomach. They were scored by examining the stomach using a binocular magnifier having a 35× magnification. The total surface of visible lesions (mm) was given a severity rating on 1-10 scale, each 4 mm^2 = 1 scale.

Data are presented as mean ± SEM of 10 animals per group. The means were compared by the analysis of variance. Differences were considered significant if the p value was less than 0.05.

RESULTS

Ethanol at the dose of 1 ml/rat and the concentration of 60%, administrated p.o., produced gastric mucosal ulceration in 100% of animals with a mean ulcer index of 5.8/10. Diazoxide prevented the appearance of ethanol lesion. This was significant only at the highest dose (45 mg/kg) ($p < 0.01$). There was no significant change in the fasting blood glucose level in groups treated with diazoxide compared to rats treated with ethanol alone (Table 1). Three Different doses of glibenclamide aggravated gastric lesions. This was neither dose dependent nor significant compared to ethanol treated rats (Table 2). The FBS of the glibenclamide treated animals was significantly lower than controls. Rats treated with different doses of diazoxide, glibenclamide or enalapril alone showed no gastric ulceration (table 1-3).

Enalapril prevented EIGU. This was significant only with the dose of 20 mg/kg of the drug (Table 3).

Table 1. Effects of diazoxide on ulcer index and fasting blood sugar (FBS) in different animal groups and controls. All drugs were used at the dose of 1 ml/100 g of body weight. Values are mean ± SEM.

Drugs	*Ulcer Index (0-10)*	*FBS (mg/kg)*
Saline	0.00 ± 0.00	65.60 ± 6.08
Ethanol 60% **(E)**	5.80 ± 0.59	104.8 ± 9.26
Diazoxide 5 mg/kg + **E**	4.80 ± 0.51	105.9 ± 9.73
Diazoxide 15 mg/kg + **E**	4.67 ± 0.67	91.29 ± 13.6
Diazoxide 45 mg/kg + **E**	2.65 ± 0.53*	105.6 ± 8.33
Diazoxide 5 mg/kg	0.15 ± 0.12	78.50 ± 6.20
Diazoxide 15 mg/kg	0.00 ± 0.00	80.22 ± 7.10
Diazoxide 45 mg/kg	0.00 ± 0.00	92.15 ± 4.85

* ($p < 0.01$) significantly different from ethanol treated rats.

Table 2. Effects of glibenclamide on ulcer index and fasting blood sugar (FBS) in different animal groups and controls. All drugs were used at the dose of 1 ml/100 g of body weight. Values are mean ± SEM.

Drugs	*Ulcer Index (0-10)*	*FBS (mg/kg)*
Saline	0.00 ± 0.00	65.60 ± 6.08
Ethanol 60% **(E)**	5.80 ± 0.59	104.8 ± 9.26
Glibenclamide 2 mg/kg + **E**	7.80 ± 0.76	67.50 ± 12.7
Glibenclamide 6 mg/kg + **E**	7.13 ± 0.89	40.71 ± 5.22*
Glibenclamide 18 mg/kg + **E**	6.20 ± 0.49	44.5 ± 10.27*
Glibenclamide 2 mg/kg	0.15 ± 0.05	37.15 ± 2.65
Glibenclamide 6 mg/kg	0.10 ± 0.05	39.10 ± 3.95
Glibenclamide 18 mg/kg	0.20 ± 0.10	43.00 ± 3.20

* ($p < 0.01$) significantly different from ethanol treated rats.

Table 3. Effects of enalapril on ulcer index in different animal groups and controls. All drugs were used at the dose of 1 ml/100 g of body weight. Values are mean ± SEM.

Drugs	*Ulcer Index (0-10)*
Saline	0.00 ± 0.00
Ethanol 60% **(E)**	5.80 ± 0.59
Enalapril 10 mg/kg + **E**	4.00 ± 0.82
Enalapril 20 mg/kg + **E**	2.11 ± 0.41*
Enalapril 40 mg/kg + **E**	4.30 ± 0.67
Enalapril 10 mg/kg	0.85 ± 0.35
Enalapril 20 mg/kg	0.95 ± 0.42
Enalapril 40 mg/kg	0.65 ± 0.34

* ($p < 0.01$) significantly different from ethanol.

DISCUSSION

Ethanol-induced gastric ulceration (EIGU) is considered to be an appropriate experimental model to study the pathogenesis of gastric mucosal ulceration. The mechanism(s) of EIGU is not fully understood.

Gastric mucosal and submucosal microcirculatory changes have been implicated in the pathogenesis of gastric ulceration [3, 10]. Many investigators believe that gastric submucosal microcirculatory disturbance is the main cause of EIGU [1, 11]. Ethanol has a direct noxious action on gastric mucosa. In addition to this direct action of ethanol on the mucosa, the stasis of blood flow would prevent the dilution and carrying away of back-diffusing ethanol, and would deprive the cells deeper in the glands of required nutrients and oxygen. Both these actions result in cell damage [11, 12].

Ethanol will diffuse through the mucosa, reach the submucosa, and then dilate the arterioles that regulate the mucosal blood flow. Szabo et al reviewed extensively the part played by the vascular changes in gastric ulceration [7]. They suggested that after intragastric administration of ethanol a rapid and time dependent release of endothelin-1 into the systemic circulation preceded the development of the hemorrhagic mucosal erosions. On the other hand, endogenous nitric oxide (NO) is thought to be the regulator of gastric mucosal haemodynamic in the resting condition and after the use of ethanol [2]. It has also a protective action against noxious agents. After ethanol administration, endothelin-1 is released which results in mucosal vasoconstriction. Under this condition, NO-induced vasodilatation and its mucosal protective action is masked and gastric erosion is produced.

Because superoxides are suggested to be responsible in the pathogenesis of EIGU, study of the relationship between NO and superoxides is of important consideration [2]. Calcitonin-gene-related-peptide (CGRP) was found to be a potent endogenous vasodilator in the stomach. Prostaglandins, histamine and some other amines have also been proposed to play a part in the ethanol vasoaction [13]. Yonei and Guth [1] demonstrated that leukotrienes are partly responsible for microcirculatory disturbances in EIGU. However, this role seems not to be an essential one. Contrary to these findings, Nagata et al [5] showed that the above chemicals do not mediate ethanol-induced vasodilation in the rat stomach. These investigators demonstrated that among the modulators of vasodilatation only adenosine seems to play an important role. Apart from the mucosal microcirculatory disturbances an increase in the vascular permeability of the gastric mucosa may also play a part in the pathogenesis of EIGU [14, 15].

Recently Doi et al showed [13] that calcitonin-gene-related-peptide (CGRP) protects the gastric mucosa against ulcerogenic stimuli (e.g. 60% ethanol). They attributed this effect, at least partly, to the activation of K_{ATP} channels in the stomach. The results of the present experiment showed that EIGU is prevented by diazoxide, a K_{ATP} channel opener. This was statistically significant with the highest dose of diazoxide (45 mg/kg). Although the 3 doses of diazoxide increased the FBS, their difference with the control group was not significant. Moreover, although the FBS of rats treated with diazoxide alone was higher than controls, no ulceration was observed in this group of animals. For these reasons the prevention by diazoxide of EIGU can hardly be attributed to changes in blood glucose level. The EIGU was aggravated by glibenclamide pretreatment. The observed aggravation is in accord with recent report of Iwata et al [8]. They attributed this effect to the blockade of the K_{ATP} channel, by glibenclamide, on vascular bed of the gastric mucosa.

In the present experiments we studied the effects of pretreatment of different doses of enalapril on EIGU. Enalapril is known to be a vasodilator. Sharifi et al [16] demonstrated that the drug dilates mesenteric small arteries through the inhibition of angiotensin converting enzyme (ACE). The fact that enalapril prevented EIGU (significant only with 20 mg/kg of the drug) confirmed the part played by changes in the microcirculation in this condition. The mechanism of the protective effects of diazoxide, a potent K_{ATP} opener, on EIGU is not known. However, according to Doi et al the opening of K_{ATP} channels should be mediated by the activation of CGRP-1 receptors. CGRP is speculated to activate KATP channels in the vascular bed by the activation of adenylyl cyclase or the release of NO from the endothelium and finally counteracting the mucosal ischemia. The participation of K_{ATP} channel openers in defense of the gastric mucosa, independent of its vascular action is another possibility which is worthy of future investigation.

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