

## INTRAPERITONEAL ADMINISTRATION OF SALICYLATE DOSE-DEPENDENTLY PREVENTS STRESS-INDUCED ULCER FORMATION IN RATS

*Akçahan Gepdiremen<sup>#</sup>, Halis Süleyman*

Atatürk University, Medical Faculty, Department of Pharmacology, TR-25240 Erzurum, Turkey

*Intraperitoneal administration of salicylate dose-dependently prevents stress-induced ulcer formation in rats.* A. GEPDIREMEN, H. SÜLEYMAN, Pol. J. Pharmacol., 2003, 55, 209–212.

Stress has an important role in the induction of gastroduodenal injury. It was reported that oxygen free radicals played a role in the pathogenesis of this injury. Although some other antioxidant compounds and calcium channel blockers were examined in ulcer models, salicylate has not been tested for its gastroprotective effect in ulcer models by now. In the present study, intraperitoneal administration of 10, 25 and 50 mg/kg of salicylate dose-dependently prevented ulcer formation in obligatory immobilization model in rats. This protective effect of salicylate was found more potent than that of ranitidine for all doses tested. As expected, peroral (by gavage) administration of salicylate at 50 mg/kg exacerbated the ulcer score, in comparison with the control.

**Key words:** *salicylate, stress ulcer, free radicals*

---

<sup>#</sup> *correspondence*; e-mail: akcahan@atauni.edu.tr

## INTRODUCTION

Aspirin irreversibly acetylates cyclooxygenase, thus inactivating this enzyme and blocking prostaglandin synthesis. Aspirin is rapidly deacetylated by esterases in the body, yielding salicylate, which has anti-inflammatory, antipyretic, and analgesic effects [8]. The upper gastrointestinal side effects of aspirin are also largely the result of prostaglandin suppression in the gastrointestinal tract. Non-steroidal anti-inflammatory drugs (NSAID) cause endoscopically visible peptic ulcers in up to 20–25% of patients on chronic NSAID therapy [15]. On the other hand, salicylate has a well-known antioxidant property. Hydroxyl free radicals, which are generated *via* the iron-catalyzed Haber-Weiss reaction [2], or alternatively, *via* nitric oxide-related mechanisms [7], react with salicylate and generate 2,3- and 2,5-dihydroxybenzoic acids (DHBA). The formation of DHBA after systemic administration of salicylate is used as an index of  $\cdot\text{OH}$  generation in many tissues [13]. The free radical scavenging properties of quercetin,  $\alpha$ -tocopherol, nifedipine and tetracycline in ethanol-induced gastric mucosal injury were tested and found to have cytoprotective and gastric ulcer healing action [16]. Melatonin, a potent antioxidant compound, was found to cause a significant reduction in gastric ulceration induced by restraint immobilization at low temperatures [10]. Involvement of oxygen free radicals in the pathogenesis of stress-induced gastric mucosal injury has been shown. Extracellular glutathione and its interorgan metabolism was demonstrated to play a critical role in the protection of gastric mucosa, particularly when animals were challenged with various stressors [9]. Glutathione had also decreasing effect of the extent of ethanol-induced macroscopic injury to the mucosa of the gastric body and the antrum [11]. In the present study, we tested another potent antioxidant, salicylate, administered *ip* in a stress ulcer model in rats.

## MATERIALS and METHODS

Obligatory immobilization method was applied to investigate the effect of salicylate in rats in which ulcer was produced by exposing the animals to stress. This work was carried out according to bioethical standards. Experiments were performed on 48 male albino Wistar rats weighing 200–220 g. The rats were divided into 6 groups (every group

contained 8 rats) and placed in cages. The rats were fasted and allowed to drink only water for 18 h. First group was administered 1 ml of distilled water *ip*, and the other groups were administered 10, 25, 50 mg/kg of salicylate (Sigma Co. St. Louis, USA), 50 mg/kg of ranitidine (Deva Co. Istanbul, Turkey) *ip*, and 50 mg/kg of salicylate perorally (gavage) dissolved in the same volume (1 ml for each animal) of distilled water. One hour after drug administration, the animals were kept for 24 h in a prone position, at room temperature. Finally, the animals were sacrificed with 70 mg/kg of thiopental sodium (Abbott Co. Istanbul, Turkey) *ip*, and their stomachs were immediately removed. The ulcerative zones were macroscopically evaluated and ulcerative foci were examined on a clear acetate paper. The data were translated to the percentage of total gastric area for each rat. Antiulcer effects of salicylate was compared with ranitidine and control group.

The results were statistically evaluated with *t*-test for independent samples, and with Mann-Whitney U-test, and  $p < 0.05$  was accepted as statistically meaningful.

## RESULTS

Stress-induced ulcer was produced in rats using obligatory immobilization method. Hyperemia on the gastric mucosa of the control group was more pronounced than that observed in the salicylate (10, 25 and 50 mg/kg, *ip*)- and ranitidine (50 mg/kg *ip*)-administered groups. Salicylate administration at 50 mg/kg by gavage, was found to increase ulcer score in comparison with the control group as expected. Ulcers were distributed homogeneously throughout the gastric surface, forming rounded, oval and irregular mucosal defect with various size and depth. Surrounding tissues of ulcer areas were edematous. As seen in Figure 1, mean percentage of ulcer area in relation to total gastric area was:  $16.13 \pm 2.4$  in the control,  $6.18 \pm 1.8$  in ranitidine (50 mg/kg, *ip*)-treated group, and  $6.0 \pm 1.5$ ,  $4.7 \pm 1.8$  and  $2.38 \pm 1.2$  after *ip* salicylate treatment at 10, 25 and 50 mg/kg, respectively. Peroral administration of salicylate at 50 mg/kg, was found to worsen ulcer formation in comparison with the control group, and ulcer area constituted  $26.2 \pm 2.7$  % of total gastric area in this group.

Intraperitoneal salicylate administration dose-dependently prevented the stress-induced ulcer formation in rats, and all its doses were found more

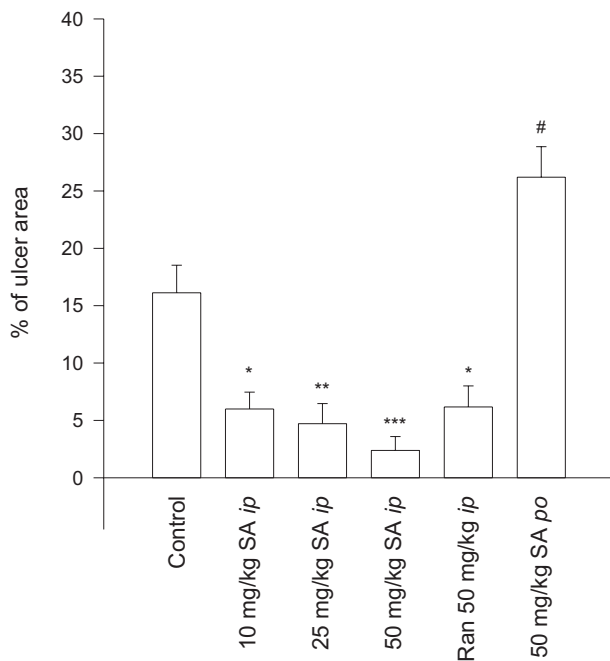


Fig. 1. The effects of salicylate (SA) administered *ip* at 10, 25 and 50 mg/kg or *per os* 50 mg/kg, and ranitidine (Ran) 50 mg/kg *ip* on stress-induced gastric ulcer formation. # and \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.005$  in respect to control

effective than 50 mg/kg of ranitidine. The most effective *ip* dose of salicylate was 50 mg/kg, while peroral administration of salicylate at 50 mg/kg worsened ulcer formation.

## DISCUSSION

Stress has an important role in the induction of gastroduodenal injury [12]. It was reported that oxygen free radicals played a role in the pathogenesis of this injury [14]. Despite some antioxidant compounds were found to have a healing effects in stress ulcer models [10, 16], there has been not much data on effectiveness of salicylate, a very potent antioxidant, in this model of ulcer, by now. Normally, prostacyclin ( $\text{PGI}_2$ ) inhibits gastric acid secretion, whereas  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  stimulate synthesis of protective mucus in both the stomach and small intestine. In the presence of aspirin, these prostanoids are not formed, resulting in increased gastric acid secretion and diminished mucus protection [8]. This effect may be caused by local action of aspirin and salicylate on the gastric mucosal cells. Cyclooxygenase inhibitors such as acetylsalicylic acid, indomethacin and sodium meclofenamate abolish high-dose DPPE-induced gastropro-

tection, whereas sodium salicylate, a lipooxygenase inhibitor, does not [5]. In the present study, when we administered salicylate by gavage, stress-induced ulcer formation was exacerbated. On the other hand, parenteral administration of salicylate dose-dependently prevented the stress-induced ulcers in rats, and this effect was found more potent than that of ranitidine.

The antiulcer activities of various selective and non-selective calcium antagonists have been widely studied [3]. Nifedipine and cimetidine were found equally effective in reducing gastric mucosal ulceration in stress-induced gastric ulcers in rats, and the effect was dose-dependent [1]. The exact mechanism by which, oxygen radicals may cause gross gastric mucosal injury is unclear. Oxidants readily react with polyunsaturated fatty acids, proteins containing sulfur amino acids and nucleic acids. This may alter vital membrane properties and damage the barrier capabilities of epithelia and endothelia [6]. Oxidants are generated in cell mitochondria by activation of generator cell receptors. Following that, increases in intracellular calcium levels activate constitutive nitric oxide synthase and it produces nitric oxide and L-citrulline from L-arginine [4]. Intracellular calcium elevation has a pivotal role in this cascade. Blocking stress-induced gastric ulcers by several calcium channel blockers may be a clue for the role of oxidant generation in this model in rats. That hypothesis becomes stronger in the light of successful blockade of stress-induced ulcer by several antioxidants such as quercetin,  $\alpha$ -tocopherol, nifedipine, tetracycline, melatonin and glutathione [10, 11, 16]. Gastroprotective effect of parenteral salicylate, probably originated from its antioxidant property. It was found that, besides sodium salicylate and high doses of aspirin in peroral administration, other salicylate-type drugs such as diflunisal, 4-aminosalicylic acid, 2,4-dihydroxybenzoic acid and methyl salicylate, and several non-acidic compounds such as proquazone, benzydamine and paracetamol were gastroprotective in ethanol-induced rat gastric injury, and this protection was found independent of prostaglandin formation [17].

Accordingly, *ip* salicylate might produce its stress ulcer preventing effect by the same mechanism in the present study. Peroral administration of salicylate exacerbated stress-induced ulcers in rats as expected. This effect might result from the

blockade of the local prostaglandin synthesis in epithelia and endothelia of gastric tissues.

### REFERENCES

1. Al-Mashhadani W.M., Karim K.H., al-Taie R.I., al-Zahawi H.M.: Nifedipine versus cimetidine in prevention of stress-induced gastric ulcers in rats. *Eur. J. Pharmacol.*, 1991, 192, 117–121.
2. Aust S.D., Chignell C.F., Bray T.M., Kayanaraman B., Mason R.P.: Contemporary issues in toxicology: free radicals in toxicology. *Toxicol. Appl. Pharmacol.*, 1993, 120, 168–178.
3. Birg N.A.: The clinico-histochemical validation of the differential use of calcium antagonists in gastroduodenal ulcers. *Terapeut. Arkh.*, 1994, 66, 48–51.
4. Faraci F.M., Brian J.E.: Nitric oxide and the cerebral circulation. *Stroke*, 1994, 25, 692–703.
5. Glavin G.B., Gerrard J.M.: Characterization of the gastroprotective effects of N,N-diethyl-2-[4-(phenylmethyl)phenoxy]-ethanamine hydrochloride, a non-H1/non-H2 histamine antagonist. *Digestion*, 1990, 47, 143–148.
6. Halliwell B., Gutteridge J.M.C.: *Free Radicals in Biology and Medicine*, Clarendon, Oxford, 1985.
7. Hammer B., Parker W.D. Jr., Bennett J.P. Jr.: NMDA receptors increase OH radicals in-vivo by using nitric oxide synthase and protein kinase C. *NeuroReport*, 1993, 5, 72–74.
8. Harvey R.A., Champe P.C.: *Pharmacology*, Lippincott-Raven, New Jersey, 1997.
9. Hirota M., Inoue M., Ando Y., Hirayama K., Morino Y., Sakamoto K., Mori K., Akagi M.: Inhibition of stress-induced gastric injury in the rat by glutathione. *Gastroenterology*, 1989, 97, 853–859.
10. Khan R., Morley B.S., Daya S., Potgieter B.: The effect of melatonin on the formation of gastric stress lesion in rats. *Experientia*, 1990, 46, 88–89.
11. Loguercio C., Taranto D., Beneduce F., del Vecchio Blanco C., de Vincentiis A., Nardi G., Romano M.: Glutathione prevents ethanol-induced gastric mucosal damage and depletion of sulfhydryl compounds in humans. *Gut*, 1993, 34, 161–165.
12. Mac Dermott B.L.: *Understanding Basic Pharmacology*, F.A. Davis, Philadelphia, 1994.
13. Obata T., Yamanaka Y.: Intracranial microdialysis of salicylic acid to detect hydroxyl radical generation by monoamine oxidase inhibitor in the rat. *Neurosci. Lett.*, 1995, 188, 13–16.
14. Pieri C., Marra M., Moroni F., Recchioni R.: Melatonin: a proxyl radical scavenger more effective than vitamin E. *Life Sci.*, 1994, 55, 271–276.
15. Silverstein F.E.: Improving the gastrointestinal safety of NSAIDs. The development of misoprostol – from hypothesis to clinical practice. *Digest. Dis. Sci.*, 1998, 43, 447–458.
16. Suzuki Y., Ishihara M., Segami T., Ito M.: Anti-ulcer effects of antioxidants, quercetin, alpha-tocopherol, nifedipine and tetracycline in rats. *Jpn. J. Pharmacol.*, 1998, 78, 435–441.
17. Trautmann M., Peskar B.M., Peskar B.A.: Aspirin-like drugs, ethanol-induced rat gastric injury and mucosal eicosanoid release. *Eur. J. Pharmacol.*, 1991, 201, 53–58.

*Received: March 12, 2002; in revised form: June 17, 2002.*