

Abdurrahman ÖNEN¹
Zeki KANAY²
Cihat GÜZEL³
Doğan KURT²
Kader CEYLAN³

The Effects of Allopurinol on Stomach Mucosal Barrier of Rats Subjected to Ischemia-Reperfusion

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Abstract: The present experimental study was carried out on rats subjected to ischemia and reperfusion. The effects of allopurinol on mucus, an important component of the stomach mucosal barrier, were investigated. Twenty-one Swiss Albino rats, each weighing 200-250 g, were used. On the control group, no treatment was performed, while the sham operation group was subjected to 30 minutes of ischemia and 20 minutes of reperfusion. Then, stomachs were examined for ulcerative lesions, and ulcer scores were recorded. Mucus content was determined by the Corne

method. In the sham group, the mucus decreased to a significant extent ($p<0.01$). It was observed that allopurinol prevented stomach lesions ($p<0.01$). Also, allopurinol prevented mucus reduction in rats subjected to ischemia and reperfusion ($p<0.01$). The results indicate that allopurinol is effective on stomach mucosal barrier parameters and in preventing stomach lesions caused by ischemia and reperfusion.

Key Words: Ischemia and reperfusion, stomach mucosal barrier, allopurinol

Departments of ¹Pediatric Surgery, ³Physiology,
Faculty of Medicine
²Department of Physiology, Faculty of Veterinary
Medicine, Dicle University, Diyarbakır-Turkey

Introduction

The stomach mucosal barrier, which protects the inside of the stomach against aggressive factors, is composed of mucus, phospholipids and bicarbonate (1,2). The gastrointestinal mucosa is one of the organs most sensitive to ischemia. Due to the exposure of the gastrointestinal tract to ischemia-reperfusion and thereby the destruction of its secretion and absorption, some damage leading to mucosal necrosis occurs (3). Both free radicals, formed as a result of ischemia and reperfusion, and acid that occurs in the stomach cause erosion and ulceration in the gastric mucosa (4). Xanthine oxydase (XO) and activated polymorphonuclear leukocytes (PMN) in tissues are indicated as the primary origins of free radicals (4-7). In some studies, it was determined that allopurinol reduced the damage of gastrointestinal mucosa by inhibiting xanthine oxydase (8,9). Cross et al. stated that mucus played an important role as an antioxidant in the gastrointestinal system (10). The aim of the present study was to investigate the effects of allopurinol, which reduces damage in stomach mucosa after ischemia and reperfusion by inhibiting the xanthine oxydase, on mucus.

Materials and Methods

In this experimental study, 21 Swiss albino rats, each weighing 200-250 g, were used. They were individually placed in stainless steel cages and were kept at a controlled temperature (22°C) under a photo period of 12 h light and 12 h dark. The rats were divided into 3 groups. No treatment was performed on the control group ($n=7$). The sham operation group was subjected to 30 minutes of ischemia and 20 minutes of reperfusion ($n=7$). Twenty-four and 48 hours before the operation, a total of 100 mg/kg (2 doses) of allopurinol was given to the allopurinol group ($n=7$) by orogastric tube. Twenty-four hours after the last dose, rats were subjected to 30 minutes of ischemia and 20 minutes of reperfusion. After 24 hours of starvation, all rats were anesthetized with 50 mg/kg ketamine hydrochloride. The bottom wall was shaved and cleaned with antiseptic solution and opened with a 3 cm middle-line incision. The esophagus and pylorus were clamped with bulldog clamps. The celiac artery was subjected to 30 min of ischemia at a point 0.5cm distal from the branch to the aorta by clamping with an atraumatic vein clamp. After 30 minutes, the clamp was opened, and reperfusion was induced for 20 minutes. Then, the stomach was removed

and divided into 2 sections by the large curvature, and examined macroscopically for gastric mucosal damage. Each lesion was measured along its greatest diameter (mm). In assessment of the size of the petechiae, 5 such lesions were considered equivalent to 1 mm ulcers. The sum of the lesion lengths in each group was divided by the number of rats in that group and expressed as the mean ulcer scores (11). The amount of stomach mucus was determined by the Corne method (12). The glandular portion of the stomach was excised, weighed and immersed for 2 h in Alcian blue solution. The excess dye was removed by 2 successive rinses of 15 min each in sucrose solution. The mucus bound dye was extracted by immersing the gastric tissue in $MgCl_2$ solution, which was intermittently shaken for 1 min at 30 min intervals over a 2 h period. The blue extract thus obtained was shaken with diethylether. The resulting emulsion was centrifuged and the optical density of the aqueous phase was measured at 600 nm in a Beckman spectrophotometer. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated from standard curves.

The Mann Whitney U test was used for statistical evaluation of the results.

Results

In the control group, the mucus content was found to be $71.71 \pm 4.46 \mu\text{g/g}$ wet tissue. In sham operation group, the mucus content was found to be $50.09 \pm 5.09 \mu\text{g/g}$ wet tissue (Table). It was also determined that ischemia and reperfusion decreased the mucus content ($p<0.01$). In the allopurinol group, the mucus content was found to be $67.71 \pm 5.49 \mu\text{g/g}$ wet tissue (Table). Allopurinol significantly inhibited the decrease in mucus content caused by ischemia and reperfusion ($p<0.01$). The ulcer scores for the sham group and for the

allopurinol group were found to be $12.14 \pm 2.41 \mu\text{g/g}$ wet tissue and $7.71 \pm 0.95 \mu\text{g/g}$ wet tissue, respectively (Table). According to these results, allopurinol clearly decreased the ulcers and acute gastric erosion that developed after ischemia and reperfusion. In addition, allopurinol considerably decreased the ulcers and acute gastric erosion that developed following ischemia and reperfusion.

Discussion

It has been determined that mucosal destruction occurs during reperfusion rather than the ischemic period (13). The lipid peroxidation that occurs in the mitochondrial membrane results from the cytotoxic effects of free radicals and plays an important role in the formation of gastric lesions. Xanthine oxydase (XO) in tissues is considered the primary origin of these free radicals (5,14).

It has been determined that allopurinol, as an XO inhibitor, prevents the formation of toxic oxygen radicals, especially during the reperfusion period, and by acting as a scavenger, it makes the formed toxic oxygen radicals ineffective (13,14). Pery et al., in their studies, found that allopurinol decreased stomach lesions (15). In other studies, it was determined that allopurinol decreased the mucosal damage that occurred during reperfusion by inhibiting the formation of XO (8,9). According to the results of this study, we determined that allopurinol significantly inhibited the decrease in mucus content, as well as gastric ulcers resulting from ischemia and reperfusion.

Sugars such as glucose and mannitol are strong eliminators of the hydroxyl radical ($OH_{\cdot}$). In the mucus structure, there are some sugars, such as N-acetil-glucosamine, galactose, and fucose. Mucus is considered an antioxidant because of this feature (10). Matthew et

Groups	Ulcer scores (mm)	Mucus($\mu\text{g/g}$ wet tissue)
Control Group (n=7) (not subjected to ischemia and reperfusion)	—	71.71 ± 4.46
Sham Op. Group (n= 7) (subjected to ischemia and reperfusion)	12.14 ± 2.41	50.09 ± 5.09
Allopurinol Group (n=7) (subjected to ischemia and reperfusion)	7.71 ± 0.95	67.71 ± 5.49

Table: Allopurinol's effects on gastric mucosal mucus and ulcer index of rats subjected to ischemia and reperfusion.

al. determined that XO, formed as a result of reperfusion, decreased the viscosity of mucus, while allopurinol inhibited the decrease in the viscosity of mucus by inhibiting XO (16). Zollei determined that allopurinol, an inhibitor of xanthine oxidase, and SOD pre-treatment were useful against hemorrhagic shock and reperfusion-induced gastric mucosal lesions (17). In conclusion, we

are of the opinion that allopurinol, as an XO inhibitor, decreases the erosions that occur as a result of ischemia and reperfusion, and also maintains the mucus content, which is an important component of the stomach mucosal barrier; however, we believe that more detailed studies should be carried out to determine this mechanism.

References

1. Aase S. Disturbances in the balance between aggressive and protective factors in the gastric and duodenal mucosa. *Scand J Gastroenterol* 24:17-23,1989.
2. Allen A, Leonard JA. The mucus barrier: Its role in gastroduodenal mucus protection. *J Clin Gastroenterol* 10 (Suppl. 1): 593-98,1988.
3. Yasue N, Chan ET, Kaplowitz N, Guth PH. Effect of phorone and Allopurinol on Ischemia-Reperfusion Injury in Gastrointestinal mucosa of the rat. *Pharmacology* 44:334-343,1992.
4. Tanaka J, Yuda Y. Role of lipid peroxidation in gastric mucosal lesions induced by ischemia-reperfusion in the pylorus-ligated rat. *Biol Pharm Bull* ; 16 (1):29-32,1993.
5. Andrews FJ, Malcontenti C, Obrein, PE. Sequence of gastric mucosal injury following ischemia and reperfusion (role of reactive oxygen metabolites). *Dig Dis Sciences* 37(9): 1356-1361,1992.
6. Granger DN, McCord JM, Parks DA et al. Xanthine Oxidase inhibitors attenuate ischemia-induced vascular permeability changes in the cat intestine. *Gastroenterology* 90: 80, 1986.
7. Smith GS, Mercer DW, Cross JM, Barreto JC, Miller TA. Sucralfate attenuates gastric mucosal lesions and increased vascular permeability induced by ischemia and reperfusion in rats. *J Gastroenterol Hepatol.* 12(7): 481-9, 1997.
8. Krasna IH, Lee RT. Allopurinol protects the bowel from necrosis caused by indomethacin and temporary intestinal ischemia in mice. *J Pediatr Surg.* 28(9): 1175-1177,1993.
9. Megison MS, Horton JW, et al. Prolonged Survival and decreased mucosal injury after low-dose enteral allopurinol prophylaxis in mesenteric ischemia. *J Pediatr Surg.* 25(8): 917-921,1990.
10. Cross CE, Hallywell B, Allen A. Antioxidant Protection: A function of tracheo-branchial and gastrointestinal mucus. *Lancet.* 1328-1330,1984.
11. Ogle CW, Cho CH, Tong MC, Koo MW. The influence of verapamil on the gastric effects of stress in rats. *Eur J Pharmacol.* Jun 19; 112(3): 399-404,1985.
12. Corne SJ, Morrissey SM, Woods RJ. A method for quantitative estimation of gastric barrier mucus. *J Physiol* 242: 1169-79, 1974.
13. Parks DA, Granger DA. Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* ; 250: 749,1986.
14. Vaughan WG, Horton JW, Walker PB. Allopurinol prevents intestinal permeability changes after ischemia-reperfusion injury. *J Ped Surg* ; 27 (8): 968-973,1992.
15. Pery MA, Wadhwa S, Parks DA, Pickard W, Granger DN. Role of oxygen radicals in ischemia-induced lesions in the cat stomach. *Gastroenterology* ; 90: 362-67,1986.
16. Grisham MB, Von Ritter C, Smith BF, Lamont JT, And Granger DN. Interaction between oxygen radicals and gastric mucin. *Am J Physiol* 253 (Gastrointest. Liver Physiol. 16): 693-696,1987.
17. Zollei I. Experimental study of hypovolaemic shock-induced gastric mucosal lesions in the rat. *Ann acad Med Singapore.* 28(1): 85-9, 1999.