Scripta Scientifica Medica, vol. 34 (2002), pp 25-30

OXIDATIVE STRESS IN GASTRIC MUCOSA IN EXPERIMENTAL MODELS OF ACUTE ULCEROGENESIS. EFFECT OF PARACETAMOL AND PROPACETAMOL

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ABSTRACT

The role of the oxidative stress in acute experimental ulcerogenesis with different pathogenesis and the effect of paracetamol and propacetamol were investigated. The study was performed on male Wistar rats (220-250g) body weight. Gastric ulcer was induced by cold restraint stress (CRS, 4 hours, 4°C), acetyl salicylic acid (ASA, 300mg/kg, 0,2mL/100 g b. w., p. o.) and absolute ethanol (1ml /animal, p. o.). For the assessment of the ulcerogenesis a mean ulcer area and as markers for the oxidative stress malon dialdehyde (MDA), reduced glutathione (GSH) and uric acid (UA) levels in mucosa were used. The mean ulcer area was 101,4, 13,6 and 9,31mm² for ethanol, CRS and ASA models. Lipid peroxidation evaluated as MDA level was activated strongly in CRS and ASA and mildly in ethanol-induced ulcerogenesis. The GSH was significantly higher in CRS and ASA models. Paracetamol (250mg/kg) and propacetamol (500mg/kg) exerted antiulcerogenic effect in the three experimental models, more pronounced for paracetamol. Their protective effect was strongest (68% and 85%, respectively) for absolute ethanol. Paracetamol and propacetamol decreased MDA in gastric mucosa and sustained the elevated levels of GSH and uric acid. Gastric ulcer induced by CRS, ASA and absolute ethanol was associated with activation of lipid peroxidation. Paracetamol and propacetamol showed gastroprotective effect associated with decreased lipid peroxidation in gastric mucosa.

Key words: oxidative stress, gastric ulcer, GSH, MDA, uric acid, paracetamol, propacetamol

INTRODUCTION

Recently, there is increasing evidence that stressors of physical and chemical origin can damage gastric mucosa through enhanced production of reactive oxygen species (ROS) and oxidative stress. Such a mechanism has been shown to play an important role in gastric ulceration caused by cold restraint stress (CRS) (3), ishemia/reperfusion (5), *Helicobacter pylori* (7), non-steroidal anti-inflammatory drugs (NSAID) (6), and ethanol (10,11). Others do not establish any relationship between ROS production and gastric ulceration (22).

The para-aminophenolic analgesic-antipyretic paracetamol is one of the widely used non opioid analgesic-antipyretic for oral and rectal use. Propacetamol, as water soluble pro-drug of paracetamol, was recently introduced in the clinical practice for postsurgical pain relief. In vivo propacetamol is rapidly converted to paracetamol and therefore is suggested that the toxicity of propacetamol is almost identical to that of paracetamol. Paracetamol exerts pro-oxidant action due to its reactive metabolite N-acetyl-p-benzo quinone imine (NAPQI). On the other hand, in experimental and clinical studies, it has been proved that paracetamol protects the gastric mucosa against water-immersion and ethanol-induced stress (14,18).

In order to clarify the effect of paracetamol on the gastric mucosa and its possible relation to changes in gastric oxidative status, we investigated the effects of paracetamol and its pro-drug propacetamol on rat gastric mucosa damaged by acetyl salicylic acid (ASA). As biochemical markers of oxidative stress we determined the concentrations of the end product of the fatty acid peroxidation malondialdehyde and the endogenous antioxidant defenders glutathione and uric acid in the gastric mucosa.

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MATERIAL AND METHODS

A. Ulcer induction

Male Wistar rats weighing 220-250g were used. They were deprived of food 24h before the experiment, but had free access to water. Ulcer induction was performed by cold restraint stress (CRS) (immobilization for 4 hours at 4°C) and by oral application of the ulcerogenic agents ASA of (300mg/kg applied in a volume of 0,2mL/100g b. w.) and absolute ethanol (1ml per animal). Propacetamol was ap-

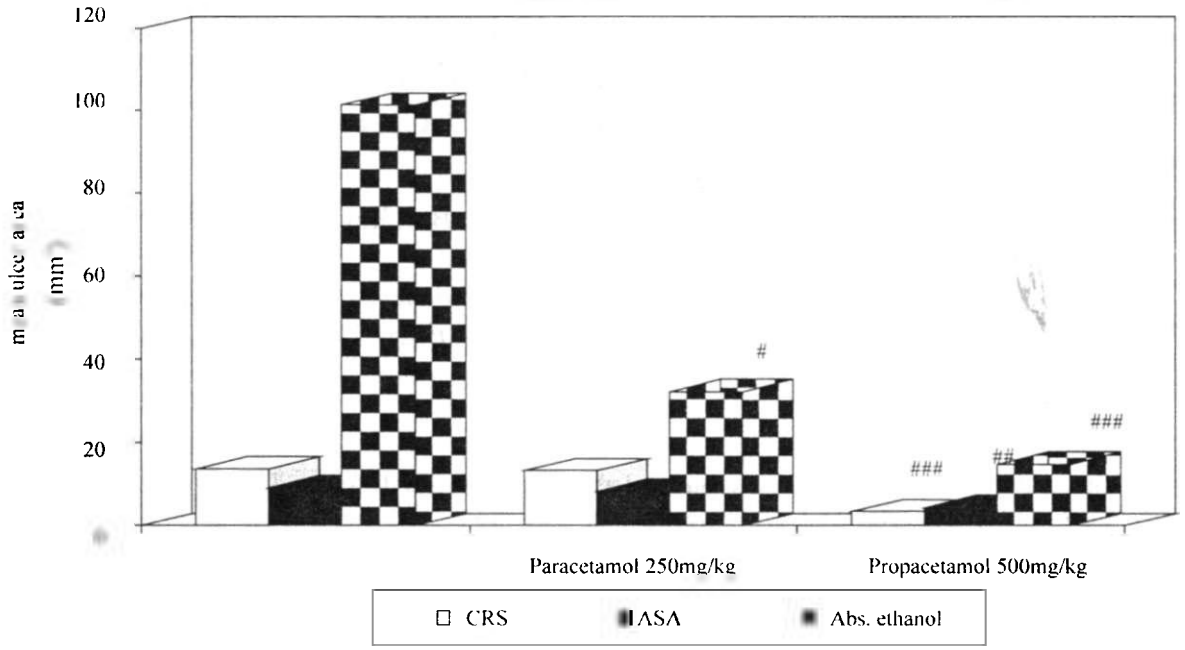

Fig. 1. Mean ulcer area in experimental models of acute ulcerogenesis. Effect of paracetamol and propacetamol. #p<0,05; ##p<0,01; ###p<0,001 in comparison to CRS, ASA and absolute ethanol groups

plied in a dose of 500mg/kg corresponding to paracetamol of 250mg/kg, the latter shown to exert a gastroprotective effect (7,18). The substances were given p. o. in a volume of 0,2 mL100/g 1 hour before ulcer induction.

Control animals were given distilled water instead paracetamol and propacetamol in the same test schedule and conditions. Each experimental group consisted of at least 6 animals.

The animals were sacrificed by rapid decapitation and exsanguination 4h after CRS or ASA administration and 1 hour after absolute ethanol application. The stomach was removed immediately, opened along the greater curvature, gently washed in physiological salt solution, spread over a pad and observed macroscopically for appearance of mucosal lesions. The length of each lesion was measured. In the case of petechia, 5 of them were considered as a 1mm^2 lesion. Mean ulcer area (mm^2) was calculated.

B. Biochemical examination

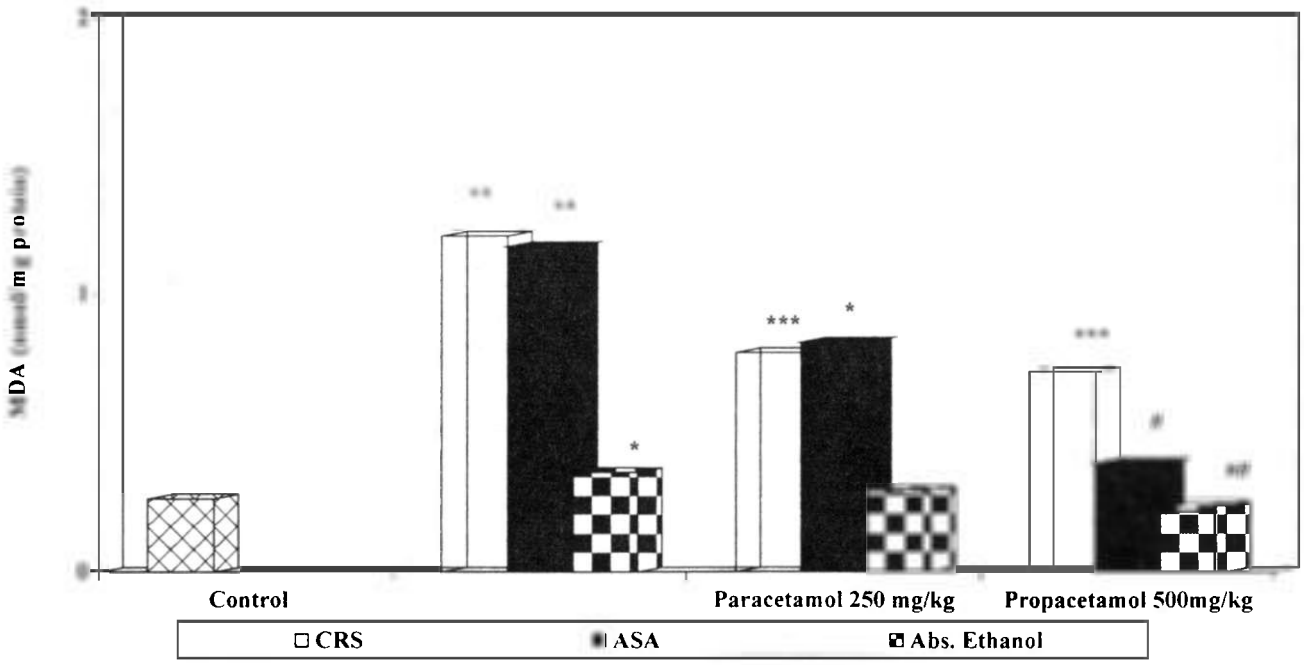

*Fig. 2. MDA in gastric mucosa in experimental models of acute ulcerogenesis. Effect of paracetamol and propacetamol. * p<0,05 , ** p<0,01, *** p<0,001 comparet to the controls; #p<0,05, ## p<0,01, ###p<0,001 compared to the CRS, ASA and absolute ethanol groups*

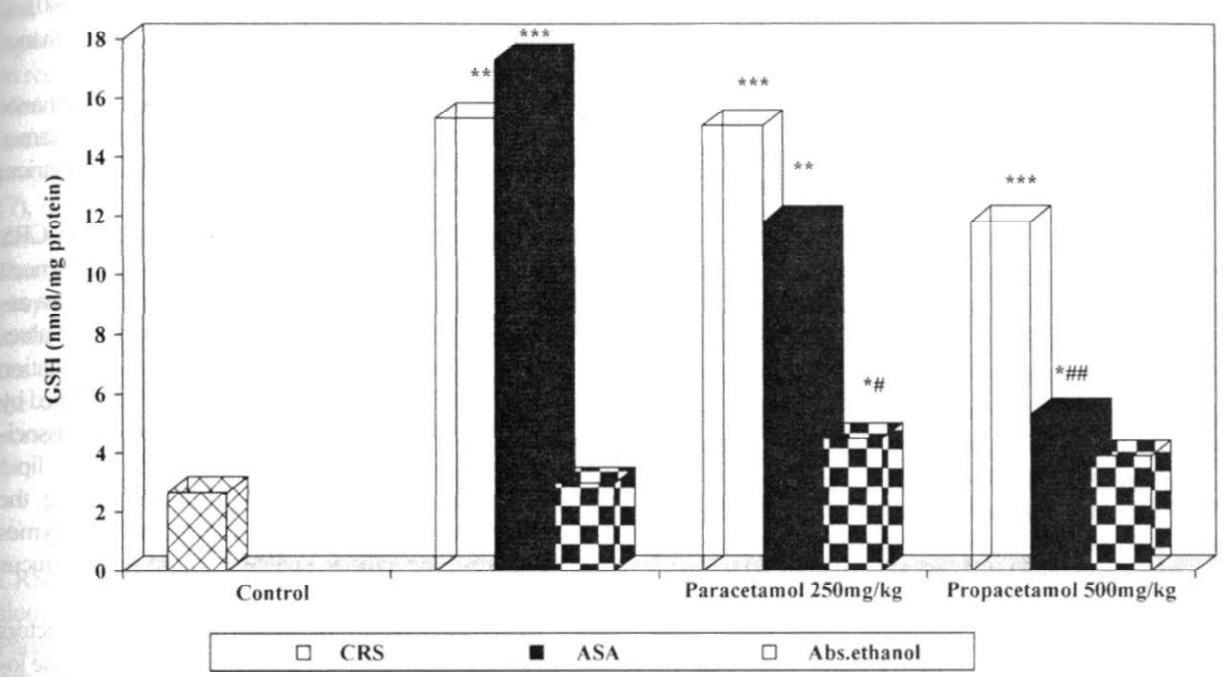

Fig. 3. GSH in gastric mucosa in experimental acute ulcerogenesis. Effect of paracetamol and propacetamol.
** p<0,05; **p<0,01; ***p<0,001 vs control; #p<0.05; ##p<0,01; ###p<0,001 vs CRS, ASA and abs. ethanol groups*

Gastric mucosa was gently separated from the underlying tissue, homogenized in 1:5w/v 50mM phosphate buffer (pH=7,4) containing 0,1 mM EDTA, at 4000rpm for 10min. The homogenate was centrifuged at 2000rpm/15 min for discarding the sediment and the supernatant was frozen until analysis. All manipulations were performed at 4-8°C. Membrane lipid peroxidation was monitored by malondialdehyde (MDA) measured by its thiobarbituric acid (TBA) reactivity in gastric mucosa homogenates, using the method of Porter *et al.* (19). Glutathione (GSH) content was assayed according to the method of Hissin and Hilf (12) using o-phthaldialdehyde as a fluorescent agent. Standard solutions of GSH were applied to calculate the glutathione content in gastric mucosal homogenates. Uric acid (UA) in gastric mucosal homogenate was determined by the method of Bergmeyer (2) based on the ability of urate to reduce phosphowolframic acid in alkaline solution to a blue coloured product.

C. Statistical analysis

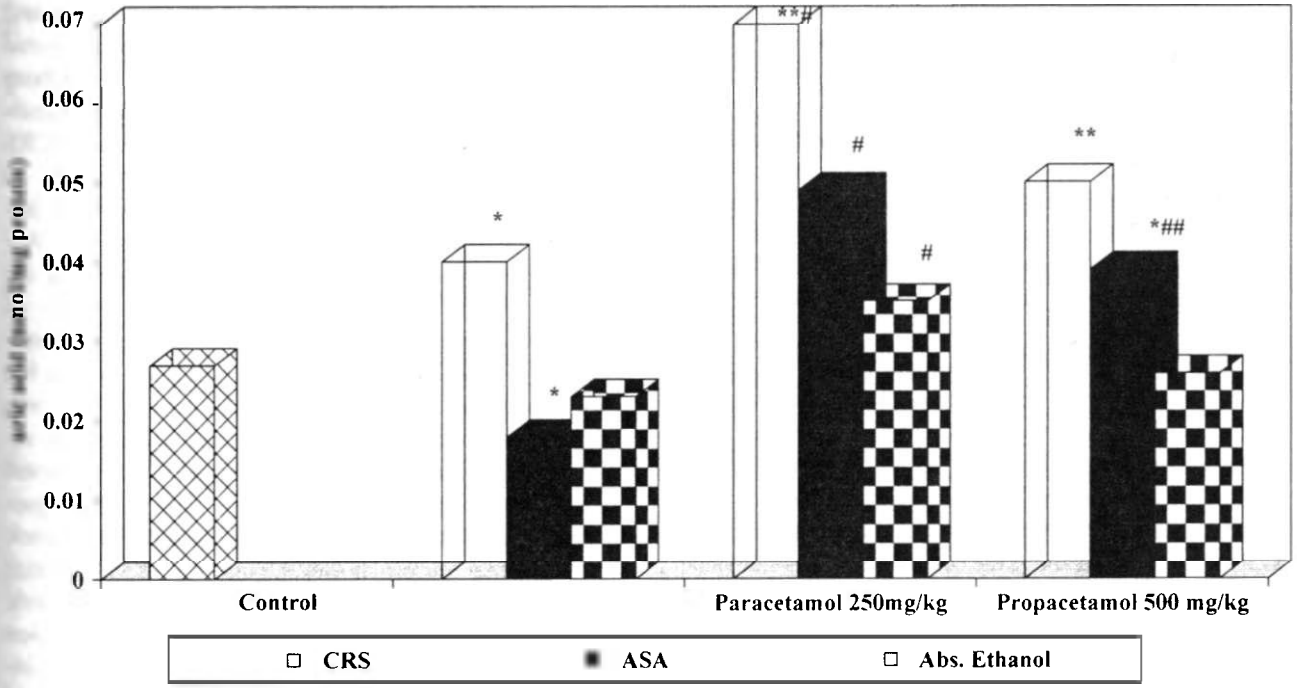

Fig. 4. Uric acid in gastric mucosa in experimental acute ulcerogenesis. Effect of paracetamol and propacetamol.
** p<0,05 ** p<0,01vs control; # p<0,05 ## p<0,01 vs CRS, ASA and absolute ethanol groups*

Data were analyzed statistically by one-way analysis of variance (ANOVA) and expressed as mean ±SEM. A value of $p<0,05$ was considered statistically significant. The statistical procedure was performed with GraphPad InStat software.

RESULTS AND DISCUSSION

A. Effect of paracetamol and propacetamol on CRS, ASA and absolute ethanol-induced gastric mucosal damage

The macroscopic observation showed that CRS, ASA and ethanol induced multiple gastric mucosal lesions, varying in number and severity. One hour after ethanol application a severe gastric mucosal damage was observed, with large areas of focal hyperemia and haemorrhagic lesions. ASA induced multiple gastric mucosal lesions most often 1-2mm in size or petechial, bleeding at the moment of the observation. CRS induced multiple mucosal lesions varying in number and size. The area of involvement was confined to the glandular part of the stomach. The mean ulcer areas were 13,6±2,3mm^2 (CRS), 9,10±0,82mm^2 (ASA) and 101,40±13,34mm^2 (absolute ethanol) (Fig. 1).

Pretreatment with paracetamol of 250mg/ kg changed the ulcer area by -3% for CRS, -10% for ASA and -68% ($p<0,05$) for ethanol model, respectively. The pretreatment with propacetamol of 500mg/kg resulted in a more pronounced decrease in ulcer area: -76% ($p<0,001$) for CRS, -53% ($p<0,01$) for ASA and -85% ($p<0,001$) for ethanol (Fig. 1).

B. Biochemical examination

In the CRS and ASA groups gastric mucosal MDA increased by 4-fold versus controls ($p<0,01$) (Fig. 2). In the ethanol group the elevation was milder - by +33% ($p<0,05$). In the CRS and ASA groups pretreated with paracetamol of 250mg/kg, MDA levels markedly decreased by 33% ($p<0,001$) and 29% ($p<0,05$), respectively, but still remained higher in comparison to the controls. In the ethanol group pretreatment with paracetamol of 250mg/kg diminished the MDA levels down to the control value. MDA level was reduced more pronounced by propacetamol of 500mg/kg by 42% in the CRS group, 67% in the ASA group ($p<0,05$ vs. the group treated only with ASA) and by 50% in the ethanol group ($p<0,01$ vs. the group treated only with ethanol) (Fig. 2).

CRS and ASA strongly increased gastric GSH concentrations (by 6-fold), while absolute ethanol did not change gastric mucosal GSH levels (Fig. 3). The increased values of GSH remained significantly higher than the control ones ($p<0,001$). In the CRS and ASA groups pretreated with paracetamol of 250mg/kg and propacetamol of 500mg/kg GSH was enhanced above the control values ($p<0,001$ and $p<0,01$, respectively).

CRS increased gastric mucosal UA by 33% ($p<0,05$), while ASA and ethanol tended to decrease UA levels (Fig. 4). In the groups pretreated with paracetamol of 250mg/kg and propacetamol of 500mg/kg, UA was enhanced above the control values and significantly higher ($p<0,05$ and $p<0,01$, respectively) than the values in the CRS, ASA and ethanol group.

In the present work we found that CRS, ASA and ethanol caused multiple gastric mucosal lesions and paracetamol and propacetamol tended to reduce the morphometrical signs of gastric mucosal damage.

The involvement of extensive lipid peroxidation in CRS and ASA-induced gastric mucosal damage was evidenced by the strong accumulation of MDA in gastric mucosa associated with a rise in glutathione values. Increased values of MDA (+50%) as an index of activated lipid peroxidation in CRS and ASA-induced ulcerogenesis was established by other authors (3,4). Probably, the ulcerogenesis is associated with oxidative injury of gastric mucosa by lipid hydroperoxides, hydroxyl radicals generated during the stress followed by inactivation of gastroprotective enzymes such as peroxidase, prostaglandine synthase, and of mucus biosynthesis (4).

In ASA and ethanol ulcerogenesis another injuring factors are inhibition of mucosal prostaglandines (ASA) and the local injuring effect. The significant elevation of GSH and UA in CRS and ASA as well as the increased MDA values may be due to the activation of free radical processes. In the ethanol model GSH and UA levels are almost unchanged corresponding to the milder elevation of MDA.

Reduced glutathione is accepted as a major mechanism for cellular protection and gastroprotection against agents generating oxidative stress. The mucosal levels of GSH are in dependence on de-novo biosynthesis and extracellular transport (24). Gamma-glutalyl cystein syntethase is the main emzyme for biosynthesis of GSH which is induced by lipid hydroperoxides (20). It was suggested that this is a possible mechanism for cellular adaptation to oxidative stress (15).

UA is capable of inactivating ROS as hydroxyl radical and hydrogen peroxide produced by the xantinoxidase activated under oxidative stress (1). On the other hand, it has been proved that there are elevated UA levels in the gastrointestinal mucosa (9). Probably, the increased urate levels in CRS are associated with activation of free radical processes.

Activation of neutrophils and NADPH-oxidase is an important element in ASA-induced ulcerogenesis (22). According to some authors (13), UA is responsible for the in-situ inactivation of the generated ROS. A gastroprotective effect of paracetamol has been demonstrated in different models of acute gastric ulcerations but the underlying mechanism remains unclear. In water-immersion stressed rats paracetamol significantly reduced gastric mucosal damage associated with promoted production of PGE2 in gastric mucosa and decreased gastric and plasma glutathione levels (18). In human gastric and duodenal mucosa, however, paracetamol was reported significantly to reduce PG production without any gastric mucosal injury, ulceration or bleeding (14). Protection against ischemia/reperfusion-induced gastric injury and inhibition of gastric lipid peroxidation was already reported (17). Our previous investigations showed that paracetamol

its pro-drug propacetamol protected gastric mucosa against CRS and prevented gastric lipid peroxidation (8). In recent years, after the introduction of propacetamol in the clinical practice a few studies dealing with its pro- or antiulcerogenic effect were performed. Having in mind that propacetamol in vivo is rapidly hydrolized to paracetamol (7), it seems logic to suggest that propacetamol will exert almost the same effect on gastric mucosa as paracetamol. Paracetamol and propacetamol decreased MDA level, but propacetamol diminished MDA in a manner corresponding to the reduction of the lesion area and maintained high values of glutathione. These findings allow the suggestion that the tested agents diminished lipid peroxidation and supported the defense reaction in response to oxidative stress.

CONCLUSION

CRS, ASA and absolute ethanol induce gastric mucosal lesions associated with increasing MDA in gastric mucosa. Paracetamol and propacetamol reduce gastric musocal injury and this effect is accompanied by significant decrease of the gastric lipid peroxidation evaluated as MDA-generation and by increased values of glutathione. Taking into consideration the role of lipid peroxidation in NSAID-gastric injury, the interference with gastric oxidative status could be suggested as a possible way through which paracetamol and propacetamol exert protective effect against CRS, ASA and ethanol-induced gastric ulceration.

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