OXIDATIVE STRESS IMPLICATIONS IN EXPERIMENTAL GASTRIC ULCER INDUCED BY INDOMETHACIN

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Abstract: In the complex aetio-pathogenesis of gastric ulcer; aggression factors; defence mechanisms and cell-protective factors are involved. Among the aggression factors; the reactive oxygen species can be found as causal agents but especially as factors that aggravate the primary lesions. The authors studied the implications of the oxidant /antioxidant system in acute ulcerations of gastric mucosa; induced by non-steroid anti-inflammatory drugs (indomethacin). An experimental model was realized using Wistar rats that received 50 mg/kg of indomethacin in 5 ml distilled water; through gavage. The gastric lesions were quantified and their histopathologic examinations were performed. From the stomach homogenate; the authors determined the parameters of the oxidative stress (lipid peroxides; free malondialdehyde); the antioxidant capacity (hydrogen donor capacity) and nitric oxide.

Significant increases of the oxidative stress parameters and significant decreases of the antioxidant capacity were found. The nitric oxide did not present significant modifications.

The administration of indomethacin produced gastric ulcerations in which the oxidative stress is implicated.

INTRODUCTION

Gastro-duodenal pathology; due to its frequency; gravity and bio-psycho-social implications; represents an important issue in medical practice. In the pathogenesis of the gastric ulcer some factors intervene: aggression factors; defence and cell-protective factors. These can be found in all people; but the difference between the healthy ones and those who develop an ulcer is the balance between aggression and defence; a balance that is in “perfect” equilibrium in former; but is unbalanced in the latter.

In the extremely complex aetio-pathogenesis of the gastric ulcer; among the aggression factors; the reactive oxygen species (ROS) can be found as either causative agents or factors that aggravate the primary lesions. In other words; the reactive oxygen species represent the cause or the consequence of some pathological changes; but in any case they can constitute a tissue aggression mechanism.

It’s a well-known fact that ROS are involved in the aetio-pathogenesis of the inflammatory and ulcerative lesions of the gastrointestinal tract. They determine: lipid peroxidation; lesions of the cellular membranes; deteriorations of the epithelial basal membrane; cellular metabolism changes; acute gastric mucosa lesions and; also; a delay in the healing of the gastric ulcerations.

Gastric and intestinal mucosa epithelium is the target of a continuous aggression induced by the reactive oxygen species generated in lumen. In physiological conditions; mucus is a very important protective mechanism that keeps the integrity of the epithelium. In the
presence of “large quantities” of free radicals; the mucus changes its rheological qualities due to a reduction in its viscosity.

In the aetio-pathogenesis of the ulcer; the ROS involvement was demonstrated through clinical and experimental work. Lipid peroxidation plays a major role in the aetio-pathogenesis of the gastric lesions induced by stress; ethanol; and non-steroid anti-inflammatory drugs (NSAID). The “stress” ulcer in rats exposed to low temperatures can be reduced through administration of the antioxidant drugs; gastric lesions found in haemorrhagic shock are induced by the reactive oxygen species; the experimental gastric ischaemia induces gastric mucosa lesions; which can be reduced by the administration of some antioxidants: superoxide-dismutase (SOD); allopurinol.

The aim of this experimental study was to estimate oxidative stress involvement in indomethacin-induced gastric ulcer.

MATERIAL AND METHOD

Adult male Wistar rats (weight 180±20 g) were used in the experiment. They had been kept for a week before the experiment in an environment with controlled temperature of 23°C±2°C; with 12 hours of light and 12 hours of darkness. Twenty-four hours prior to the experiment; the animals did not receive any food. In the day of the experiment; their weights had been measured and; through gavage; they received 50 mg indomethacin/kg body weight in distilled water. This dose was determined by probing.

The animals had been killed by cervical vertebra dislocation. The stomach was taken; dissected along the great curvature and washed in saline. Gastric mucosa was inspected using an optical microscope with 10x objective. The gastric lesions were quantified as follows: 0 = less than 3 pinhead ulcerations; 1 = more than 4 pinhead ulcerations; 2 = 1-5 ulcerations with a diameter larger than 2 mm; 3 = more than 5 ulcerations with a diameter larger than 2 mm; 4 = one or more gigantic ulcer with a diameter larger than 2 mm [1]. Gastric ulcerations index was calculated using the formula:

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UI (ulcer index) = \frac{\text{score}_{\text{animal} 1} + \text{score}_{\text{animal} 2} + \ldots + \text{score}_{\text{animal} 10}}{\text{number of animals}}
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During quantification; the samples were kept on ice. The ulcer and the periulcer areas were excised and frozen at -80°C until they had been used for measurement of oxidative stress parameters. Fragments of stomach were also immersed in formol 10% for histopathological examination.

The stomach homogenate was prepared using Polytron PT 1200E; in Tris solution 50 mM; pH = 7.6.

The following parameters of the oxidative stress were determined in gastric homogenate: free malondialdehyde (MDA) using Esterbauer method [2] and lipid peroxides through Satoh method [3]; the results being expressed in nmol/mg protein. The concentration of the proteins in the stomach homogenate was determined by Bradford method [4]. For estimating the antioxidant capacity; we measured the hydrogen donor capacity using the Janaszewska method. The hydrogen donating capacity depends on the concentrations of glutathione; cysteine; vitamin C and glucose in the biological samples. The results are expressed in percentage [5]. To determine the hydrogen donor capacity; the stomach homogenate has been brought to a protein concentration of 5 mg/ml. The nitric oxide (NO) was determined through the Griess method [6].

The results were compared to those of a control group that received physiological salt; instead of indomethacin; through gavage.
RESULTS AND DISCUSSIONS

In the control group; the ulcer index was equal with zero: none of the animals presented macroscopic or microscopic lesions of the gastric mucosa. All animals in the test group (animals that received indomethacin) presented gastric ulcerations; varying from pinhead to large ulcerations and/or complete lesion of the mucosa with haemorrhage. The histological examinations confirmed the presence of the gastric ulcerations of different diameters. (Fig.1; Fig.2).

![Fig.1. Gastric mucosa ulceration (Haematoxilin-eosin; 20x).](image1)

![Fig.2. Inflammatory infiltrate in gastric mucosa (Haematoxilin-eosin; 50x).](image2)

The test group presented increased values of the lipid peroxides; dosed in stomach homogenate (0.78 ± 0.16 nmol/mg) in comparison with the control group (0.3 ± 0.02 nmol/mg) (Fig. 3).
Malondialdehyde presented also significantly increased values in animals that received indomethacin (0.28 ± 0.02 nmol/mg) as compared to controls (0.17 ± 0.02 nmol/mg) (Fig.4).

The total local antioxidant capacity was studied following the hydrogen donors in the stomach homogenate. The test group (with indomethacin) presented significant decrease of the hydrogen donors’ capacity (17.26 ± 6.61 %) in comparison with the control group (28.62 ± 1.67 %) (Fig. 5).
These results are in accordance to those in the literature showing the involvement of the oxidative stress in the gastric ulcer induced by the NSAID administration.

Nitric oxide showed a tendency to decrease in indomethacin group (0.19 ± 0.11 nmol/mg) as compared to the control group (0.29 ± 0.21 nmol/mg) (Fig.6); but the decrease was not significant.

Fig. 5. Hydrogen donor capacity (in gastric homogenate) in animals treated with Indomethacin; in comparison with the witness group.

Fig. 6. The nitric oxide in gastric homogenate in rats treated with Indomethacin; compared to the witness group.
CONCLUSIONS

Experimental and clinical studies suggested that the reactive oxygen species and the reactive nitrogen species (RNS) have an important role in the aetio-pathogenesis of the inflammation and ulceration of the digestive tract. The acute lesions of gastric mucosa; induced by NSAID administration; represent an important issue in clinical practice. Medical literature provides data that indicate the involvement of the reactive oxygen species in producing the NSAIDS-induced acute gastric ulcerations; frequently complicated with superior digestive haemorrhage. With this respect; it has been showed that ROS produced by the activated neutrophils and by the xantin-xantinoyxdase system have an important role in the pathogenesis of the gastric lesions induced by ibuprofen [7].

The gastric lesions appeared after the administration of piroxicam or meloxicam are also produced by the decrease of superoxide-dismutase and by the modification of glutathione homeostasis in gastric mucosa [8].

The inhibition of prostaglandin-synthetase by NSAID seems to be the biochemical element that produces the gastric lesions [9]; as the endogenous prostaglandin has a major role for maintaining the integrity of the gastric mucosa; inhibiting the hydrochloric acid; stimulating the secretion of mucus and of bicarabonic anions. [10]

The oxidative stress is implicated in the production of the acute gastric ulcerations after the administration of indomethacin [11]. In rats; the administration of 25-100 mg/kg of indomethacin produces acute gastric ulcerations; concomitant with the increase of lipoperoxidation; the decrease of the activity of SOD; glutathione- peroxidase and of glutathione-transferase [12].

The apple extract; reach in poliphenols; administrated in animals with gastric ulcer experimental induced by indomethacin; prevents the gastric mucosa lesions; probably because of the antioxidant effect [13].

The endogenous nitric oxide has protective effects -modulates the vascular tonus and the blood flow in gastric mucosa. The medications that generate nitric oxide protect the gastric mucosa. If the NO synthesis is inhibited; gastric lesions can appear. The healing of the acute gastric lesions depends on the blood flow in the ulcer line; the substances that reduce this flow (the inhibitory factors of the prostaglandin synthesis –indomethacin) delay the healing process. Nitric oxide has cytotoxic effects on the gastric mucosa: if it is released in large quantities; it can have noxious effects on the local circulation producing low blood pressure; decreasing the sensitivity at endogenous vasocostriction substances and haemorrhage.

In our study; gastric ulcerations; varying in size; were present in all animals which were given indomethacin and were accompanied by a reduction in antioxidant capacity and an increase in lipid peroxidation markers in gastric homogenate. This findings support the idea of oxidative stress involvement in indomethacin-induced gastric ulcer. Even if it was not significant; we found a tendency for NO to decrease in indomethacin treated animals.

The mechanism by which ROS and RNS intervene in the production and/or in the aggravating of the acute gastric ulcerations induced by NSAID are still ambiguously; so that; the experimental models can be useful in studying of the aetio-pathogenesis mechanisms implicated in gastric ulcerations.

BIBLIOGRAPHY


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