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Adaptation of rat gastric tissue against indomethacin toxicity☆

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ABSTRACT

Indomethacin is used in the treatment of inflammatory diseases. But the drug toxicity limits its usage. This study investigated whether adaptation occurred after various dosages of repeated (chronic) indomethacin in rats to the gastro-toxic effects of indomethacin. It also examined whether the adaptation was related to oxidant–antioxidant mechanisms and oxidative DNA damage in gastric tissue. To illuminate the adaptation mechanism in the gastric tissue of rats given various dosages of chronic indomethacin, the levels of oxidants and antioxidants (GSH, MDA, NO, SOD and MPO), activities of COX-1 and COX-2 enzymes and oxidative DNA damage (8-OHd Gua/10^5 Gua) were measured. Results were compared to 25-mg/kg single-dose indomethacin group, and the role of oxidant and antioxidant parameters and oxidative DNA damage in the adaptation mechanism was evaluated. The average ulcer areas of gastric tissue of the 0.5-, 1-, 2-, 3-, 4-, and 5-mg/kg dosages of chronic indomethacin given to rats were 19.5 ± 3.7, 12.5 ± 3.3, 10 ± 5.2, 4.5 ± 3.6, 8.6 ± 2.4, and $9.5 \pm 2.1\,mm^2$, respectively. This rate was measured as $21.3 \pm 2.6\,mm^2$ in the single-dose indomethacin group. Consequently, after various dosages of repeated (chronic) indomethacin administration in rats, it was observed that a clear adaptation developed against gastric damage and that gastric damage was reduced. The best adaptation was observed in the gastric tissue of the 3-mg/kg chronic indomethacin group. In parallel with the damage reduction, the oxidant parameters (MDA and MPO) and oxidative DNA damage (8-OHd Gua/10^5 Gua) were reduced, and the antioxidant parameters (GSH, NO and SOD) were increased. There is no relation between COX enzymes and adaptation mechanism. This circumstance shows that not COX-1 and COX-2 enzymes, oxidant and antioxidant parameters may play a role in the adaptation mechanism.

1. Introduction

Indomethacin, an indole acetic acid derivative non-steroidal anti-inflammatory drug (NSAID), is used in the treatment of inflammatory diseases, such as rheumatoid arthritis, osteoarthritis, gut arthritis, burst, tendinitis, traumatic synovitis, and ankylosing spondylitis [1]. However, the drug's toxicity is a big problem [2] and limits usage [3].

The most common toxic effect of indomethacin is gastrointestinal tract (GIT) damage; the toxic effects vary from unknown blood loss to ulcer perforation [3,4]. Since indomethacin is an ulcerogenic substance [5], it is frequently used in the development of experimental ulcer models [6,7]. It is well-known that the toxic effect of indomethacin in rat gastric tissue is more severe than that of other NSAIDs [8]. Studies have showed that both anti-inflammatory effect and gastro-toxic effect of NSAIDs is linked to cyclooxygenase (COX) enzyme inhibition [9]. Inhibition of COX-2 is responsible from the therapeutic (anti-inflammatory) effect of NSAIDs and inhibition of COX-1 is responsible from gastrointestinal toxic effect of them [1,10]. It is well-known that indomethacin inhibits both of them. It was reported that this detrimental effect of indomethacin was linked to prostaglandin (PG) synthesis (especially PGE2) inhibition [11]. COX is an enzyme that plays a role in the PG pathway. In light of this idea, we decide to determine the COX-1 and COX-2 enzyme activities as an indicator of PGE2.

Many methods were developed for preventing NSAIDs' toxic effects on GIT. For this reason, nitric oxide releasing NSAIDs (NO-NSAIDs) have been produced [3]. In addition, some investigations have shown that GIT tissue can adapt to the toxic effects of chronic administered drugs and have supposed that this phenomenon, adaptation, may be useful in the decrease of the toxic effects of drugs [12–14]. In the literature, we found some evidence that human gastric tissue can adapt to the toxic effects of indomethacin [15]. But in animals this evidence is controversial. Kuwayama et al. have shown that rat gastric tissue adapts to 5 mg/kg of indomethacin [16]. But other literature has indicated that in animals there is no adaptation to 5 mg/kg or higher doses of

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indomethacin [14,17,18]. In addition, despite some literature that shows indomethacin adaptation [16], adaptation-dose correlation and mechanisms that play a role in the adaptation phenomenon have not been indicated.

The significant difference between the oxidant–antioxidant parameters of damaged and healthy tissue [19–21] means that oxidation may play a role in the adaptation phenomenon. For this reason, in this investigation we aimed to define in which dose, chronic administered indomethacin would developed the best adaptation in rat gastric tissue, and to show the relation between adaptation-dose correlation and oxidant–antioxidant parameters, COX-1/COX-2 enzymes and oxidative DNA damage.

2. Materials and methods

2.1. Chemicals

Whole biochemical assay compounds were purchased from SIGMA Chem. Co. (Germany) and MERCK (Germany). In addition, indomethacin was bought from Deva Drugs (Turkey), and thiopental was obtained from IE Ulagay (Turkey).

2.2. Animals

A total of 48 male albino Wistar rats weighing 200–220 g were obtained from the Ataturk University Medicinal and Experimental Application and Research Center. The animals were divided into treatment groups before the experimental procedures were initiated. The animals were housed and fed under standard conditions in a laboratory where the temperature was kept at 22 °C. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by the local animal care committee of Ataturk University.

2.3. Indomethacin ulcer test

In this experiment, the 48 rats were divided into 8 groups, each consisting of 6 rats. The first 6 groups were chronic indomethacin groups, the 7th group was the 25 mg/kg single-dose indomethacin group, and the 8th group consisted of healthy intact rats. Doses of 0.5, 1, 2, 3, 4, and 5 mg/kg/day of indomethacin were administered daily by oral gavage to the first 6 groups for 14 days. During this period, the single-dose indomethacin group and the healthy intact rats were given the same volume of distilled water orally. After the last administration, the rats were fasted for 24 h. At the end of this period, a dose of 25 mg/kg of indomethacin was applied by oral gavage to all groups except the healthy intact rats. Distilled water was administered to the healthy intact rats. Six hours after the administration of indomethacin, all rats were sacrificed using a high dose (50 mg/kg) of thiopental. The stomachs were removed, and the ulcer focus on the gastric surface was assessed macroscopically. Ulcer areas were measured by millimetric paper [22]. Results obtained from the groups given chronic indomethacin were compared to those of the single-dose indomethacin group. After this assessment, the stomachs were transported to the biochemistry laboratory for determination of the oxidant–antioxidant parameters and oxidative DNA damage.

2.4. Biochemical analysis of gastric tissue

In this part, 0.2 mg of whole gastric tissue (damaged and healthy parts together) was weighed for each stomach. The samples were homogenized in ice with 2-ml buffers (consisting of 0.5% HDTMAB [0.5% hexa desil tri methyl ammonium bromide] pH 6 potassium phosphate buffer for myeloperoxidase analyze, consisting of 1.15% potassium chloride solution for malondialdehyde analysis and pH 7.5 phosphate buffer for the other analyses). Then, they were centrifuged at 4 °C, 10,000 rpm for 15 min. The supernatant part was used as the analysis sample.

2.5. Total glutathione (tGSH) analysis

The amount of GSH in the total homogenate was measured according to the method of Sedlak and Lindsay with some modifications [23]. The sample was weighed and homogenized in 2 ml of 50 mM Tris–HCl buffer containing 20 mM EDTA and 0.2 mM sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 ml of 25% trichloroacetic acid, and the precipitate was removed after centrifugation at 4200 rpm for 40 min at 4 °C and the supernatant was used to determine GSH level. 1500 μl of measurement buffer (200 mM Tris–HCl buffer containing 0.2 mM EDTA at pH 7.5), 500 μl supernatant, 100 μl DTNB (10 mM) and 7900 μl methanol were added to a tube and vortexed and incubated for 30 min in 37 °C. 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) was used as an chromogen and it formed a yellow-colored complex with SH groups. The absorbance was measured at 412 nm using a spectrophotometer. The standard curve was obtained by using reduced glutathione.

2.6. Superoxide dismutase (SOD) analysis

Measurements were performed according to Sun et al. [24]. When xanthine is converted into uric acid by xanthine oxidase, SOD forms. If nitro blue tetrazolium (NBT) is added to this reaction, SOD reacts with NBT and a purple-colored formazan dye occurs. The sample was weighed and homogenized in 2 ml of 20 mM phosphate buffer containing 10 mM EDTA at pH 7.8. The sample was centrifuged at 6000 rpm for 10 min and than the brilliant supernatant was used as assay sample. The measurement mixture containing 2450 μl measurement mixture (0.3 mM xanthine, 0.6 mM EDTA, 150 μM NBT, 0.4 M Na_2CO_3, 1 g/l bovine serum albumin), 500 μl supernatant and 50 μl xanthine oxidase (167 U/l) was vortexed. Then it was incubated for 10 min. At the end of the reaction, formazan occurs. The absorbance of the purple-colored formazan was measured at 560 nm. As more of the enzyme exists, the least $O_2^{\bullet -}$ radical that reacts with NBT occurs.

2.7. NO (nitric oxide) analysis

Nitric oxide levels were measured by the Griess reaction [25,26]. Nitric oxide measurement is difficult because of its brief half-life. Therefore, nitrate and nitrite levels, which are stable end products of nitric oxide metabolism, were used. The measurement mixture [100 μl sample, 100 μl NADPH (50 μmol/l), 100 μl FAD (5 μmol/l), 20 μl nitrate reductase (200 U/l)] was prepared and incubated for 20 min in 37 °C. Then 25 μl $ZnSO_4$ (300 g/l) added to this mixture and by this way deproteinization occurred. This mixture centrifuged for 15 min at 1000 rpm. The supernatant part was used as measurement assay. 100 μl Griess reagent and 100 μl metaphosphoric acid were added to the supernatant and a deep purple azo compound occurred. The Griess reagent consists of 0.5 g sulfanilamide, 12.5 g phosphoric acid and 0.05 g N-(1-napthyl)-ethylenediamine in 500 ml distilled water. The method is based on a two-step process. At the first step, nitrate is converted nitrite by nitrate reductase. At the second step, nitrite reacts with the Griess reagent and at the end of this reaction a deep purple azo compound occurs. The absorbance of this deep purple azo compound was measured at 540 nm wavelength by photometric measurement. This azo chromophore accurately determines nitrite concentration as a marker of NO.