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Effects of Nonsteroidal Antiinflammatory Drugs on the Thiol Groups and Lipid Peroxidation in Ethanol-Induced Oxidative Stress

Nonsteroidal Antienflamatuvar İlaçların Etanol'de Oluşturulan Oksidatif Streste Lipit Peroksidasyon ve Tiyol Grupları Üzerine Etkileri

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Abstract

The aim of this study was to investigate whether nonsteroidal antiinflammatory drugs (NSAIDs) such as ibuprofen (IBU), indomethacin (INDO), aspirin (ASP), and celecoxib (CEL) could enhance the susceptibility the oxidative stress caused by the acute intoxication with ethanol in gastric mucosa, liver and brain of mice. The results showed that only the IBU treatment contribute to the control of the lipid peroxidation (LPO) and thiol group levels in all tissues. However, CEL can be able to increase the susceptibility of gastric mucosa to ethanol-induced oxidative stress like classic NSAIDs, while it has a significant protective effect in brain tissue..

Key words: Nonsteroidal antiinflammatory drugs,COX-2 inhibitors, lipid peroxidation, total thiol groups, reduced glutathione.

Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are among the most widely used therapeutics primarily for the treatment of pain and inflammation. Like aspirin, all other NSAIDs such as ibuprofen, naproxen and indomethacin develop their mode of action by blocking cyclooxygenase (COX). Unfortunately, treatment with traditional NSAIDs results in a mechanism-based toxicity mainly in the gastrointestinal tract, kidney and liver and thus limit their therapeutic usefullness when long-term treatment is necessary (Allison *et al.*,1992; Mitchell 1994).

Recently, it was discovered that COX exists in two isoforms: COX-1 and COX-2. New generation NSAIDs, such as celecoxib and rofecoxib selectively antagonize COX-2 without the side effects (i.e. gastrointestinal hemorrhage and ulceration) caused by classic NSAIDs (Dannhardt and Kiefer 2001). Therapeutic effects and side effects of NSAIDs are closely related to their biochemical mechanism of action. It has been proposed that oxygen radical dependent injuries may be an important factor that lead to cellular damage induced by NSAIDs.

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Some authors emphasized the increased susceptibility of tissues to lipid peroxidation (LPO) during experimentally induced inflammatory processes (oxidative stress) as well as the inhibitory effect of NSAIDs on LPO formation (Zentella-de-Pina *et al.*, 1993; Seibert *et al.*, 1994).

At the first step of this experiment, NSAIDs namely aspirin, ibuprofen and indomethacin (non selective COX inhibitors) and celecoxib, a selective COX-2 inhibitor were assayed to search whether they can induce the susceptibility of stomach to oxidative stress caused by the acute intoxication with ethanol. Second, their capability in preventing the free radical injury in liver and brain was evaluated.

To evaluate the cellular injury, LPO was estimated as thiobarbituric acid reactive substances (TBARS) production. Total thiol groups (T-SH) and non-protein-thiol groups (NP-SH) were also determined.

Materials and Methods

Animals and Treatment: Locally bred Swiss Albino male mice (Refik Saydam Hıfzısıhha Institute, Animal Care Unit, Ankara, Turkey) weighing approximately 22g were used. The compounds , aspirin (200 mg/kg), ibuprofen (200 mg/kg), indomethacin (10 mg/kg) and celecoxib (100 mg/kg) were suspended in 0.5 % carboxymethylcellulose and administered 60 min before the absolute ethanol (0.1 ml/mice) to mice by using gastric gavage needle. One hour after the application of ethanol, under ether anaesthesia livers and brains were removed.

Lipid peroxidation in Tissues : To evaluate the LPO, TBARS were determined in tissue samples. The method is based on the formation of a red chromophore which absorbs at 532 nm, following the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) and other breakdown products of peroxidized lipids (Ohkawa et al., 1979; Jamall and Smith, 1985).

Total thiol groups (T-SH) and Non-protein thiol groups (NP-SH ;GSH) in Tissues

Tissues were homogenized in 0.02M ethylenediamine tetraacetic acid disodium salt (EDTA-Na_2). For determination of total -SH groups, aliquots of 0.5 ml of the homogenates were mixed with 1.5 ml of 0.2M Tris buffer, pH 8.2, and 0.1 ml of Ellman's reagent . The mixture was brought to 10.0 ml with 7.9 ml of absolute methanol. Color was developed for 15 min and the reaction mixtures centrifuged at approximately 3000xg at room temperature for 15 min. The absorbance of supernatants was read at 412 nm.

For the determination of nonprotein-SH groups (GSH), aliquots of 5.0 ml of the homogenates were mixed with 4.0 ml distilled water and 1.0 ml of 50% trichloroacetic acid (TCA). Tubes were centrifuged for 15 min at approximately 3000xg. Two ml of supernatant was mixed with 4.0ml of 0.4M Tris buffer, pH 8.9 and 0.1 ml Ellman's reagent was added. The absorbance was read at 412 nm against a sample blank within 5 min (Sedlak and Lindsay, 1968).

% changes were calculated according to following formula: n-n′ / n x 100

n was the average level of the control or ethanol groups

n′ was that of the test group of animals

Statistical Analysis: Statistical significance of data (expressed as mean ± SEM) was assessed by the Tukey-Kramer post hoc test using Instat computer software.

Results and Discussion

Although the pathogenesis of alcohol dependent diseases is not completely understood, an important factor contributing to alcoholic injuries may be the result of enhanced peroxidative

stress. Ethanol induced gastric mucosal damage in experimental animals is associated with an increase the LPO and a decrease in tissue levels of thiol compounds (Dutta *et al.*, 1995).
The tissue level of nonprotein sulfhydryls (NP-SH, mainly reduced glutathione, GSH) is high in organs such as liver, stomach and brain potentially exposed to free radical reactions. Since ethanol-induced gastric mucosal damage is associated with generation of toxic oxygen metabolites the possibility exists that the drop in NP-SH tissue levels is due to oxidation of GSH (Loguercio *et al.*, 1991; Yoshikawa *et al.*, 1997).
Our results indicate that ethanol, in a concentration that causes gross damage to gastric mucosa, beside increasing the TBARS level ($p<0.001$), significantly affects sulfhydryl tissue levels in the stomach, decreasing T-SH and GSH ($p<0.001$; Table1). This is in agreement with previous reports showing that ethanol-induced tissue damage is associated with a significant increase in TBARS and decrease in sulfhydryl tissue levels in the experimental animals (Pihan *et al.*, 1987; Loguercio *et al*, 1992). In addition to this finding, ASP, INDO and CEL treatment induced a great susceptibility to ethanol-induced LPO in the gastric mucosa ($p<0.05$, $p<0.01$, $p<0.01$ respectively) in this study. Amazingly, in our preliminary study, we showed that CEL (100mg/kg bd.wt/day for 5 days) did not affect the mucosal integrity and tissue level of LPO compared to control (data was not shown).
It is well known that, in contrast to COX-1, COX-2 enzyme is not found in the normal human stomach. It is found in high concentrations in sites of inflammation. COX-1 is found in the gastric mucosa and is responsible for the mucosal protection. According to our result, CEL is contraindicate with chemicals such as ethanol which can induce the gastrointestinal ulcers. It was proposed previously that, once an ulcerative injury is present, CEL can elevate the wound or injury (Dannhardt and Kiefer, 2001).

Table 1. Effects of aspirin, indomethacin, ibuprofen and celecoxib on the alteration of LPO (as TBARS levels) and thiol groups in ethanol-induced oxidative stress in the stomach of mouse.

Groups (n=5-7)	**TBARS** (nmol/g tissue)	**T-SH** (µmol/g tissue)	**NP-SH (GSH)** (µmol/g tissue)
Control	64.4±4.6	168.7±0.7	24.1±1.5
Ethanol[a]	130.9±5.2 ***	116.7±1.2 ***	6.0±0.5***
ASP + Ethanol[b]	155.2±8.5*	130.6±1.9***	8.6±0.6
INDO + Ethanol[b]	167.2±2.3**	119.3±3.2	2.7±0.5
IBU+ Ethanol[b]	139.7±2.9	124.5±2.6	10.9±0.4**
CEL + Ethanol[b]	168.3±2.6**	126.7±2.2*	7.6±0.3

a: Compared to control group; **b:** Compared to ethanol group
$p<0.05$, $**p<0.01$, $***p<0.001$

On the other hand, acute ethanol ingestion has also been reported to produce a marked decrease in hepatic GSH concentration. Most of the studies suggest that depletion of GSH following acute ethanol intoxication is a result of increased LPO (Dutta *et al.*, 1995). One possible mechanism for the increased GSH level observed during IBU treatment may be the activation of enzymes responsible for its de-novo biosynthesis from its amino acid precursors. Decrease in the tissue concentration of GSH may have significant deleterious effects on the tissue defense system against oxidants and xenobiotic substances. Treatment with ASP and IBU did not increase the susceptibility of hepatic tissue to LPO provoked by ethanol in this study (Table 2).

On the other hand, it has been clearly seen that CEL treatment prevented the increase in the TBARS level induced by ethanol in liver tissue even though it was not found statistically significant. It is concluded that some NSAIDs, especially IBU in this study contributed to controlling the LPO.

Table 2. Effects of aspirin, indomethacin, ibuprofen and celecoxib on the alteration of LPO (as TBARS levels) and thiol groups in ethanol-induced oxidative stress in the liver of mouse .

Groups (n=5-7)	**TBARS** (nmol/g tissue)	**T-SH** (μmol/g tissue)	**NP-SH (GSH)** (μmol/g tissue)
Control	220.5±4.7	324.6±7.2	38.6±1.6
Ethanol[a]	309.6±6.6***	179.6±11.2 ***	10.9±0.3***
ASP + Ethanol[b]	269.9±6.3*	229.2±11.8*	17.1±0.8**
INDO + Ethanol[b]	315.4±9.4	216.9±13.2	7.4±0.9
IBU+ Ethanol[b]	267.5±14.7*	234.7±9.6**	22.0±1.0***
CEL + Ethanol[b]	273.7±8.6	297.2±9.4***	17.4±0.9**

a: Compared to control group; **b:** Compared to ethanol group
*p<0.05, **p<0.01, ***p<0.001

The brain is particularly vulnerable to LPO, since it consumes a large amount of oxygen and is rich in polyunsaturated, highly peroxidable fatty acids. Moreover, it has only moderate levels of enzymatic and nonenzymatic scavengers of reactive species. It was shown that some NSAIDs may reduce risk for Alzheimer's Disease (Omodeo-Sale *et al.*, 1997). In ethanol treated mice, compared to the control ones, sensitivity to LPO appears to be greatly affected, showing a significant increase in TBARS production in brain tissue ($p<0.01$; Table 3).

Table 3. Effects of aspirin, indomethacin, ibuprofen and celecoxib on the alteration of LPO (as TBARS levels) and thiol groups in ethanol-induced oxidative stress in the brain of mouse.

Groups (n=5-7)	**TBARS** (nmol/g tissue)	**T-SH** (μmol/g tissue)	**NP-SH (GSH)** (μmol/g tissue)
Control	234.6±5.9	221.9±2.4	29.2±1.7
Ethanol[a]	284.2±8.6**	178.3±3.2 ***	4.5±0.5***
ASP + Ethanol[b]	321.8±12.8	195.4±2.6**	9.2±0.5
INDO + Ethanol[b]	303.9±14.5	187.3±0.8	6.0±0.8
IBU+ Ethanol[b]	197.8±7.8***	206.2±3.4***	24.0±1.1***
CEL + Ethanol[b]	204.9±5.3***	200.3±3.5***	9.8±0.6*

a: Compared to control group; **b:** Compared to ethanol group
* p<0.05, **p<0.01, ***p<0.001

Actually, IBU and CEL decreased the LPO ($p<0.001$) and increased the GSH levels in both liver ($p<0.001$, $p<0.01$ respectively) and brain ($p<0.001$, $p<0.05$ respectively) in this study. It is known that liver can generate cysteine from methionine for GSH biosynthesis and can readily export GSH for supplying extrahepatic tissues. The inhibition of LPO in brain exerted by the scavengers and the increase in tissue TBARS production after the addition of some NSAIDs, partly suggest an involvement of GSH in cellular peroxidation.

Conclusion;

1-Since ethanol-induced gastric mucosal damage is associated with generation of toxic oxygen metabolites, the possibility exists that the drop in NP-SH and T-SH tissue levels is due to oxidation of reduced glutathione and oxidation of proteins. Selective COX-2 inhibitors such as CEL, probably lead to delayed wound healing and may aggravate the injury in patients with gastric ulcer or consumption of ethanol.

2-In addition to the well known peripheral role of COX-2 in inflammation, recent and our results indicate an important role in the central nervous system.

3-Liver and mucosal injury from IBU use has rarely been reported IBU ranked lowest in overall risk. Thus, we can say that IBU is more safe than the other drugs used in this investigation, at the given dose.

Özet

Bu çalışmanın amacı, nonsteroidal antiinflamatuvar ilaçlardan ibuprofen (IBU), indometasin (INDO), aspirin (ASP) ve selekoksib (CEL)'in fare gastrik mukoza, karaciğer ve beyin dokularında, akut etanol intoksikasyonunun neden olduğu oksidatif strese hassasiyeti artırıp artırmadıklarını araştırmaktır. Çalışmanın sonuçları, sadece IBU uygulamasının tüm dokularda lipit peroksidasyon (LPO) ve tiyol grupları düzeylerinin kontrolüne katkısı olduğunu göstermektedir. Oysa, CEL beyin dokusunda anlamlı bir koruyucu etkiye sahip olduğu halde, etanolün gastrik mukozada neden olduğu oksidatif strese hassasiyeti klasik nonsteroidal antiinflamatuvar ilaçlar gibi artırabilmektedir.

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