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Protective effect of erdosteine against naphthalene-induced oxidative stress in rats

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ABSTRACT: Here, we look at how erdosteine protects against naphthalene-induced toxicity and what part free radicals play in that process. An oral dosage of 1100 mg naphthalene/kg in maize oil was administered to female Sprague-Dawley rats. Before receiving naphthalene, rats were orally administered 50 mg/kg/day of ergosteine for three days. Twenty-four hours following naphthalene delivery, the rats were beheaded. Activities of glutathione (GSH), malondialdehyde (MDA), sodium (Na+), potassium (K+) ATPase, and myeloperoxidase (MPO) were measured in liver and kidney tissues. Plasma samples were tested for total antioxidant capacity (AOC), IL-1□ , IL-6, 8-hydroxy-2'-deoxyguanosine (8-OHdG), and aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine levels, and lactate dehydrogenase (LDH) activity. After administering naphthalene, the levels of GSH, Na+, K+-ATPase activity, and plasma AOC were all significantly reduced, while the levels of MDA and MPO were significantly increased in the tissues. In addition, the naphthalene group showed a substantial rise in levels of pro-inflammatory mediators (TNF-□ , IL-□ , IL-6), 8-OHdG, LDH activity, AST, ALT, creatinine, and BUN. Conversely, erdosteine therapy averted all of these naphthalene-induced metabolic alterations. Finally, it seems that erdostein protects tissues by regulating the production of inflammatory mediators, balancing the oxidant-antioxidant state, and limiting neutrophil invasion.

KEY WORDS: Naphthalene; erdosteine; lipid peroxidation; glutathione; myeloperoxidase

INTRODUCTION

The majority of crude oils include polycyclic aromatic hydrocarbons (PAHs), which are the primary harmful and long-lasting components. Toxic PAHs have spread over aquatic ecosystems due to the disposal of petroleum products into the ocean (1). The mutagenic/carcinogenic, genotoxic, and cytotoxic effects of certain of these compounds have been established (6). Many commercial products, including mothballs, toilet scent discs, and soil fumigants, contain naphthalene, a general type of polycyclic aromatic hydrocarbons (PAHs). This compound is used in the production of synthetic resins, anthranilic and phthalic acids, and naphthylamines (7, 8).

One possible mechanism by which naphthalene's toxicity manifests is by the formation of hydroxylated compounds, such as 1-naphthol, 2-naphthol, and 1,2-di-hydroxynaphthalene, which in turn cause oxidative damage (9, 10). There is evidence that naphthalene exposure increases serum and liver lipid peroxides (11), while decreasing hepatic selenium dependent glutathione peroxidase activity (12). In both humans and rats, exposure to napthalene causes hemolytic anemia. The same was true for female Sprague-Dawley rats; when given napthalene (1100 mg/kg), lipid peroxidation in the mitochondria of the liver and brain increased 2.5-fold. 24 hours after treatment, it was shown that naphtha-lene toxicity is partially caused by free radicals and oxidative stress mediated by free radicals (8).

A new mucoactive drug called Erdosteine [N-(carboxymethylthioacetyl)-homo- cysteine thiolactone] has two sulphydryl groups that are blocked: one is in an aliphatic side-chain and the other is encased in the heterocyclic ring (13). The molecule's free radical scavenging and antioxidant capabilities are activated when the hepatic metabolism frees these chemically inhibited sulfhydryl groups (14). Evidence from many inflammatory models supports its free radical scavenging action, which in turn protects against oxidant-induced tissue damage (15– 18). A similar protective effect of erdosteine against oxidative damage to colonic tissues caused by colitis has been shown by us (19). Based on this backdrop, our goal was to explore the potential protective effects of erdosteine on the hepatic and renal tissues in rats exposed to naphthelene utilizing biochemical analysis.

MATERIALS AND METHODS

Animals

All experimental protocols were approved by the Marmara University School of Medicine Animal Care and Use Commit- tee. Female Sprague-Dawlwey rats (200-250 g) were kept at a constant temperature (22 $\pm$ 1^{o} C) with 12 h light and dark cy- cles and fed a standard

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rat chow.

Experimental Design

Rats were given orogastrically either erdosteine (50mg/kg/ml n=16) or saline (n=16) for 3 consecutive days. On the fourth day, after an overnight fasting with free access to water, half of the saline or erdosteine- treated rats were given 1100mg/kg/ ml of naphthalene in corn oil by gavage(naphtehelen groups), while the other half of the saline or erdosteine-treated group was given orogastrically corn oil (control groups). All rats were decapitated at 24 hour of naphthalene or corn oil admin- istration. After decapitation of the animals, trunk blood was collected and liver and kidney were carefully dissected and stored at –70 C^{o} for the determination of tissue malondialde- hyde (MDA) and glutathione (GSH) levels, Na^{+}-K^{+} ATPase and myeloperoxidase (MPO) activities.

Biochemical analysis

Blood urea nitrogen (20) and serum AST, ALT (21) and creati- nine (22) concentrations and LDH levels (23) were determined spectrophotometrically using an automated analyzer. Plasma levels of tumor necrosis factor alpha (TNF-□), interleukin (IL)- 1□ , and IL-6 were quantified according to the manufacturer's instructions and guidelines using enzyme-linked immuno- sorbent assay (ELISA) kits specific for the previously men- tioned rat cytokines (Biosource International, Nivelles, Bel- gium). The total antioxidant capacity in plasma were measured by using colorimetric test system (ImAnOx, cataloge no. KC5200, Immunodiagnostic AG, D-64625 Bensheim), accord- ing to the instructions provided by the manufacturer. The 8-OHdG content in the extracted DNA solution were determined by enzyme-linked immunosorbent assay (ELISA) method (Highly Sensitive 8-OHdG ELISA kit, Japan Institute for the Control of Aging, Shizuoka, Japan). These particular assay kits were selected because of their high degree of sensitivity, spe- cificity, inter- and intra-assay precision, and small amount of plasma sample required conducting the assay.

Malondialdehyde and glutathione assays

Tissue samples were homogenized with ice-cold 150 mM KCl for the determination of malondialdehyde (MDA) and glutath- ione (GSH) levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously (24). Lipid peroxi- dation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56 x 10^{5} M^{-1} cm^{-1} and results are ex- pressed as nmol MDA/g tissue. GSH measurements were per- formed using a modification of the Ellman procedure (25). Brief- ly, after centrifugation at 3000 rev./min for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4.2H_2O$ so- lution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were cal- culated using an extinction coefficient of 1.36 x 10^{4} M^{-1} cm^{-1}. Results are expressed in μmol GSH/g tissue.

Myeloperoxidase activity

Myeloperoxidase (MPO) is an enzyme that is found predomi- nantly in the azurophilic granules of polymorphonuclear leu- kocytes (PMN). Tissue MPO activity is frequently utilized to estimate tissue PMN accumulation in inflamed tissues and correlates significantly with the number of PMN determined histochemically in tissues. MPO activity was measured in tis- sues in a procedure similar to that documented by Hillegass et al. (26). Tissue samples were homogenized in 50 mM potassi- um phosphate buffer (PB, pH 6.0), and centrifuged at 41.400 g (10 min); pellets were suspended in 50 mM PB containing 0.5
% hexadecyltrimethylammonium bromide (HETAB). After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41.400 g for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB, o-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Measurement of Na^{+},K^{+}-ATPase activity

Measurement of Na^{+},K^{+}-ATPase activity is based on the meas- urement of inorganic phosphate that is formed from 3 mM disodium adenosine triphosphate added to the medium dur- ing the incubation period
(27). The medium was incubated in a 37º C water bath for 5 min with a mixture of 100 mM NaCl, 5 mM KCl,
6 mM $MgCl_2$, 0.1 mM EDTA, 30 mM Tris HCl (pH 7.4). Following the preincubation period, Na_2ATP, at a final concentration of 3 mM was added to each tube and incubated at 37º C for 30 min. After the incubation, the tubes were placed in an ice bath, and the reaction was stopped. Subsequently, the level of inorganic phosphate was determined in a spectropho- tometer (Shimadzu, Japan) at excitation wavelength of 690 nm. The specific activity of the enzyme was expressed as □ □ □ l $Pimg^{-1}$ protein h^{-1}. The protein concentration of the supernatant was measured by the Lowry method (28).

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego; CA; USA). All data were ex- pressed as means □ SEM. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Values of $p<0.05$ were regarded as significant.

RESULTS

Treatment with erdosteine resulted in a substantial decrease in both AST and ALT levels ($p<0.01$-0.05), in

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contrast to the considerably higher levels seen in the saline-treated naphthalene group (p<0.001). Similarly, the levels of BUN and creatinine, which had a substantial rise in the group treated with saline naphthalene (p<0.001- 0.01), were restored to the control values by treating with erdosteine (p<0.01-0.05). Lactate dehydrogenase in serum The naphthalene group that was treated with saline had a significantly higher nase activity, which is a symptom of widespread tissue damage (p<0.001), but this effect was avoided by the administration of erdosteine (p<0.001). When compared to the control group, the saline-treated naphthalene group showed a substantial increase (p<0.001) in proinflammatory cytokines TNF-
□ □ IL-1□ and IL-6. However, this increase in serum

els, as a marker of oxidative DNA damage, were significantly
higher in the saline treated naphthalene group than in control

groups (p<0.001) and erdosteine treatment reduced the

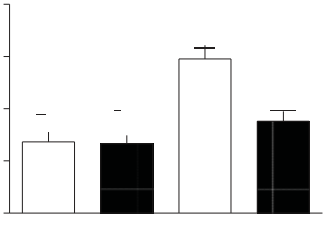
elevated
plasma 8-OHdG levels (p<0.001). The total plasma antioxidant
capacity is decreased significantly due to naphthalene adminis-
tration (p<0.01), and this decrease was prevented by

erdosteine
treatment (p<0.05, Table 1).

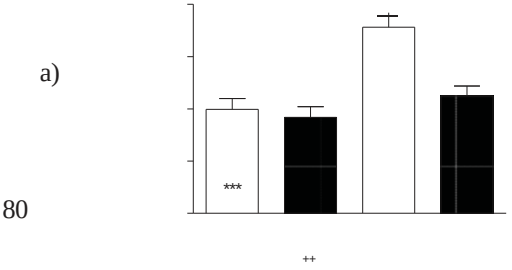
TABLE 1. Effects of erdosteine (50 mg/kg) treatment on some biochemical parameters in the serum of experimental groups. Each group consists of 8 animals. Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey's multiple comparison tests.

Parameters	Control Groups		Naphthalene Groups	
	Saline treated	Erdosteine treated	Saline treated	Erdosteine treated
AST (U/l)	246±10	250±12	368±18***	257±16++
ALT (U/l)	76.2±7.7	73.8±7.8	154±14.6***	110±6.3+
Creatinine (U/l)	0.7±0.1	0.6±0.1	1.8±0.2***	0.8±0.1+++
BUN (U/l)	25.7±2.1	23.5±2.1	40.2±2.5**	28.5±2.6++
LDH (U/I)	1799±135	1794±242	4759±243***	2416±101+++
TNF-α (pg/ml)	7.6±1.1	8.1±1.3	30.9±3.1***	13.6±2.1+++
IL-1β (pg/ml)	15.5±1.7	16.5±1.9	34.2±3.8***	18.8±2.2++
IL-6 (pg/ml)	4.9±0.9	6.5±1.2	16.1±1.5***	9.9±1.2+
8-OHdG (ng/ml)	0.8±0.1	0.9±0.1	6.6±0.9***	2.1±0.5+++
AOC (pg/ml)	447±49	424±53	187±21**	362±32+

Data are mean ± SEM. ** p <0.01, *** p <0.001 compared to saline treated control group. + p <0.05, ++ p <0.01, +++ p <0.001compared compared to saline treated naphthalene group.



cytokine levels caused by naphthalene was abolished (p<0.001) after receiving erdosteine therapy. The average amount of malondialdehyde (MDA), a key byproduct of lipid peroxidation, was found to be higher in the liver and kidney tissues following naphthalene administration compared to the control groups (p<0.001). On the other hand, erdostein treatment resulted in a significant reduction of MDA levels (p<0.01, Fig. 2) in the naphthalene group.



DISCUSSION

/The results show that erdogane was able to significantly reduce napthelene-induced lipid peroxidation and neutrophil infiltration in the liver and kidney tissues, as antioxidant GSH level and
inhibited Na+-K+-ATPase activity, all thanks to its free radical scavenging capabilities. Excessive oxidative stress is also shown by decreased AOC and elevated levels of LDH, AST, ALT, BUN, creatinine, and 8-OHdG in the blood. Furthermore, the data show that erdosteine administration reversed changes in all tissue parameters, reduced naphtelene-induced oxidative stress indicators in plasma, and enhanced renal and hepatic functioning.

The most vulnerable organs to naphthalene's harmful effects in both people and animals in the lab are the lungs and the lens of the eye (29). Chronic exposure to low doses of naphtha-lene is the most common cause of human toxicities (30). Administering naphthalene *intraperitoneally at a dose of 200 mg/kg damaged the lungs of mice (30). The formation of cataracts after exposure to naphthalene +w+ as documented by Koch et al. (31). Naphthalene caused sister chromatid exchanges in ovary cells from Chinese hamsters, both with and without metabolic stimulation from outside sources. As well as increasing the frequency and severity of chronic* inflammation in the lungs and olfactory epithelial

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radical spin trapping agent) were also useful in reducing naphthalene-induced cataracts (29) and (41).
It has been shown that erdosteine and its active metabolites significantly inhibit phorbol 12-myristate 13- acetate-induced luminol dependent chemiluminescence
(42). Furthermore, erdosteine's protective effects on inflammation and management in response to hypoxia suggest that it has anti-inflammatory effects by scavenging ROS produced by inflammatory cells. Also, erdosteine protects rats against pulmonary fibrosis and other inflammatory lung disorders (43–45). Research on the protective effects of er-dosteine on rat kidneys after renal ischemia/reperfusion (I/R) has been conducted in the lab (46, 47). The rise in kidney oxidant enzyme activity (XO and myeloperoxi-dase) and MDA levels was reduced in the pretreatment group when given erdosteine at dosages of 10 mg kg−1 day−1, in comparison to the I/R group
(46). In addition, the oxidative damage to the liver and kidneys in rats might be avoided by starting oral administration of erdosteine (50 mg kg−1 day−1) two days before to intraperitoneal injection of cisplatin (48). When administered with cisplatin, erdosteine enhanced CAT, GSH-Px, and SOD activity in renal and liver tissues (49).

Lipid peroxidation is a free radical producing mechanism that may be linked to oxidant-induced tissue damage; MDA is a reliable measure of the level of lipid peroxidation (8, 50, 51). Compared to the control group, animals given napthalene had significantly higher levels of malondialdehyde (MDA) in both the liver and the kidneys. But erdosteine's antioxidant properties kept MDA levels from rising.
It is possible that neutrophil-generated free radicals contribute to this upregulation of lipid peroxidation. Because free radicals not only cause tissue damage directly, but they also seem to induce the accumulation of leukocytes in the affected tissue, which leads to tissue harm indirectly via active neutrophils. The synthesis and release of reactive oxygen metabolites and cytotoxic proteins into the extracellular fluid by activated neutrophils is known to produce tissue damage. These proteins include proteases, myeloperoxidase, and lactoferrin, among others. Neutrophils secrete MPO and other substances that harm tissues when they are triggered by certain stimuli. This makes it a measure of the amount of neutrophils in the blood. An increase in MPO activity caused by naphthalene may lead to inflammation and organ damage, since neutrophil infiltration is a crucial event in acute inflammation. However, it was shown that proinflammatory cytokines TNF-alpha, IL-1□ , and IL-6 were considerably elevated, further confirming that naphthalene toxicity is strongly associated with inflammatory processes and oxidative damage. Our data imply that the inhibition of plasma cytokines and tissue neutrophil infiltration is a mediator of the protective effects of er-dosteine, as treatment with erdosteine considerably reduced these cytokines and blocked neutrophil infiltration into the injured tissue.
Rats have also been studied to see how erdosteine affects testicular torsion and detorsion (52). Before the rats were twisted or detwisted in the left unilateral testicles, they were given Erdosteine at a dose of 50 mg kg−1 day−1 for two days. By blocking the buildup of free radicals, erdosteine administration mitigated the histological damage caused by testicular torsion and detorsion (49).
To conclude, this study shows that naphthalene oxi-dative metabolism is a major cause of organ damage. Erdosteine has protective effects because it inhibits neutrophil infiltration, balances oxidant-antioxidant status, and regulates the production of inflammatory mediators. This suggests that Erdosteine may play a role in the treatment of organ failures caused by chemical or drug toxicities in the future.

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