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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

High osmolar contrast medium causes mild oxidation in liver, bladder, and ovary tissues from rats: vitamin C has protective role

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Abstract The purpose of this study was to investigate effects of ionic high-osmolar contrast medium on oxidative metabolism in liver, urinary bladder, and ovary tissues and to obtain information about possible protective effects of vitamin C. Twenty-one female rats, 14 weeks old, were used in this study. They were divided into three groups of seven rats: Sham (group I), contrast (group II), contrast + vitamin C (group III). Vitamin C was given orally to the animals in group III during the study period. On the fifth day, contrast medium was given via intravenous infusion as a single dose to the animals in groups II and III. On the sixth day of the study, the animals were killed with anesthesia by ketamine hydrochloride. Then, their liver, bladder, and ovary tissues were removed to measure analyses parameters. Our results suggested that contrast medium led to some increases in malondialdehyde levels in the liver, bladder, and ovary tissues and that vitamin C prevented these increases in the tissues. Nitric oxide level also was found to increase in the contrast-treated animals and vitamin C prevented this increase in the liver tissue.

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Ionic high-osmolar contrast medium leads to weak oxidant stress in rat liver, bladder, and ovary tissues, and vitamin C prevents this oxidant stress.

Keywords Contrast medium · Oxidant/antioxidant status · Vitamin C

Introduction

Reactive oxygen species (ROS) leads to several diseases, such as inflammation, aging, cancer, arteriosclerosis, hypertension, and diabetes (Kang *et al*., 2006; Laurindo *et al*., 1991; Nakazono *et al*., 1991; Parthasarathy *et al*., 1992; Palinski *et al*., 1995; Darley-Usmar and Halliwell, 1996; Cooke *et al*., 1997; Farinati *et al*., 1998). Superoxide anion, hydrogen peroxide, and peroxynitrite are among ROS. Superoxide anion is generally generated by mitochondrial electron transport chain in vivo. Hydrogen peroxide is produced from superoxide anion by superoxide dismutase (SOD). Peroxynitrite is generated by the reaction of superoxide anion with nitric oxide (NO). Nitric oxide synthase (NOS) converts arginine to citrulline and NO (Murray, 2000). Under physiological conditions of the organism, NO is a nontoxic mediator and has a vasodilator effect; however, when it is formed at high rates, e.g., in areas of inflammation, it may contribute to cell and tissue damage (Rauen *et al*., 2007).

To alleviate detrimental effects of ROS, all body cells have some antioxidant enzymes, such as SOD, glutathione peroxidase (GSH-Px), catalase (CAT), and nonenzymatic antioxidants, such as vitamins C and E. Superoxide dismutase produces hydrogen peroxide from superoxide anion. GSH-Px converts hydrogen peroxide to water and CAT also converts hydrogen peroxide to molecular oxygen and water (Chan, 2001; Irmak *et al*., 2002; Miura, 2004). If this endogenous balance between oxidant and antioxidant system is destroyed, oxidative stress occurs (Miura, 2004). Lipid peroxidation end product, such as malondialdehyde (MDA), measurement is generally used for evaluating oxidative injury (Lucchi *et al*., 2005).

Ioxitalamate is a high osmolar and ionic contrast agent frequently used in imaging processes (Klingmuller, 1985). There are 650.9 mg/ml of meglumine ioxithalamate and 96.6 mg/ml of sodium ioxithalamate in Telebrix 35. Contrast media can be divided into several classes according to ionicity (ionic or nonionic), chemical structure (monomer or dimer), and osmolality. Contrast-induced nephropathy (CIN), from mild to serious level, has been reported by the use of different classes of contrast media. CIN may cause high morbidity and mortality (Sharma and Kini, 2005). Low-osmolar nonionic contrast media have been shown to have fewer nephrotoxic effects than do high-osmolar ionic contrast media (Heinrich *et al*., 2005). In the literature, there are a lot of studies about CIN. However, as far as we know, the effects of contrast medium have not fully investigated in the liver, bladder, and ovary. For this reason, this study was designed to evaluate the effects of ionic high-osmolar contrast medium and possible protective effects of vitamin C against oxidation caused by ionic high-osmolar contrast medium in rat liver, bladder, and ovary tissues.

Materials and methods

Twenty-one female Wistar-albino rats of 14 weeks old (200 ± 10 g) were used in the study. The animals were obtained from Laboratory Animals Unite of Ankara Teaching and Research Hospital. The study was approved by the Ethical Committee of Ankara Teaching and Research Hospital. As the contrast agent, Telebrix 35 (ioxithalamate meglumine + ioxithalamate) produced by *Guerbet AG* with 350 mg iodine per milliliter was used.

The animals used in the study were divided into three groups of seven rats: Sham (group I), contrast (group II), contrast + vitamin C (group III). Vitamin C was given in drinking water at the dose of 250 mg/kg per day to the animals in group III during the study period (Ueta *et al*., 2003). During the fifth day of the study, contrast medium was given to the animals in groups II and III via intravenous infusion as a single dose (8.5 ml/kg weight, approximately 3 g/kg iodine load; Lee *et al*., 2006). The animals in group I (control group) were given physiological serum solution in the same volume. On the sixth day of the study, the animals were anesthetized by intramuscular injection of 100 mg/kg of ketamine hydrochloride and killed. Subsequently, their liver, bladder, and ovary tissues were removed surgically.

The tissues were homogenized in a physiologic saline solution (1 g in 5 ml) and centrifuged at 4,000*g* for 20 min. Upper clear supernatants were removed to use in the analyses. Lowry's method was used to measure protein levels of the supernatants (Lowry *et al*., 1951). Protein values were adjusted to equal concentrations before analyses.

In the liver tissues MDA, NO levels and SOD, GSH-Px, CAT, and NOS enzyme activities were measured. In the bladder and ovary tissues, MDA levels were measured only. Approximately 1,000 μl of supernatant was used for analysis MDA, NO level and SOD, GSH-PX, CAT, NOS enzyme activities measurement (respectively, 400 μl, 100 μl, 200 μl, 100 μl, 50 μl, 200 μl).

The thiobarbituric acid reactive substances (TBARS) method was used for MDA measurement (Dahle *et al*., 1962). GSH-Px activity was measured by following changes in NADPH absorbance at 340 nm (Paglia and Valentine, 1967). CAT activity was determined by measuring the decrease of H_2O_2 absorbance at 240 nm (Aebi, 1974). In the activity calculations (IU, international unit), extinction coefficients of uric acid, H_2O_2, and NADPH were used for XO, CAT, and GSH-Px, respectively. SOD activity was measured by the method based on nitro blue tetrazolium (NBT) reduction rate. One unit for SOD activity was expressed as the enzyme protein amount causing 50% inhibition in NBT reduction rate (Durak *et al*., 1996). The total NOS activity (mIU/ml) method was based on the diazotization of sulfanilic acid by NO at acid pH and subsequent coupling to N-(1-napthyl-ethylene diamine) (Griess reaction) (Durak *et al*., 2001). The level of NO was estimated by the method based on Griess reaction. Because nitrate anion does not give a diazotization reaction with sulfanilic acid, the samples were treated by cadmium (a reducing agent) to reduce nitrate anions into nitrite anions before the NO estimation (Ridnour *et al*., 2000).

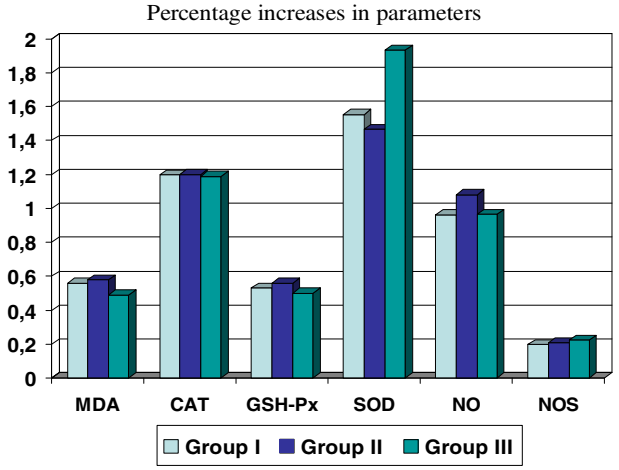
Histological investigation

For light microscope analyses, tissue samples from the liver, ovary, and urinary bladder were obtained from all animals. The samples were fixed in 10% neutral buffered formalin and then washed in flowing water. Tissues were dehydrated with rising concentrations of ethanol (50%, 75%, 96%, 100%). After dehydration, specimens were put into xylene to obtain transparency and infiltrated with and embedded in paraffin. The blocks were sectioned at 5 μm by Leica RM 2125 RT and stained for routine light microscopy, using hematoxylin and eosin (H&E) staining. Histopathologic examinations were performed and photographed by Nikon Eclipse E 600.

Results were expressed as arithmetic mean ± standard deviation (SD). For statistical evaluation of results, the Kruskal–Wallis test was used. Values of $p < 0.05$ were considered significant.

Table 1 Parameters in liver tissue (mean ± SD; $n = 7$ in each group)

Group	MDA	CAT	GSH-Px	SOD	NO	NOS
I	0.561 ± 0.063	119.6 ± 11.48	0.053 ± 0.019	155.2 ± 42.14	0.963 ± 0.108	0.197 ± 0.018
II	0.579 ± 0.129	119.8 ± 13.43	0.056 ± 0.019	146.4 ± 25.89	1.082 ± 0.359	0.208 ± 0.031
III	0.490 ± 0.004	118.9 ± 7.59	0.050 ± 0.019	193.2 ± 28.87[a]	0.965 ± 0.127	0.224 ± 0.030



Note: CAT and SOD values have been divided by 100 and GSHPx values have been multiplied by 10 in this bar graph

MDA = malondialdehyde, CAT = catalase, GSH-Px = glutathione peroxidase, SOD = superoxide dismutase, NO = nitric oxide, NOS = nitric oxide synthase

Group I: Sham-operated; Group II: contrast; Group III: contrast + Vit C

[a] II versus III; $p < 0.05$

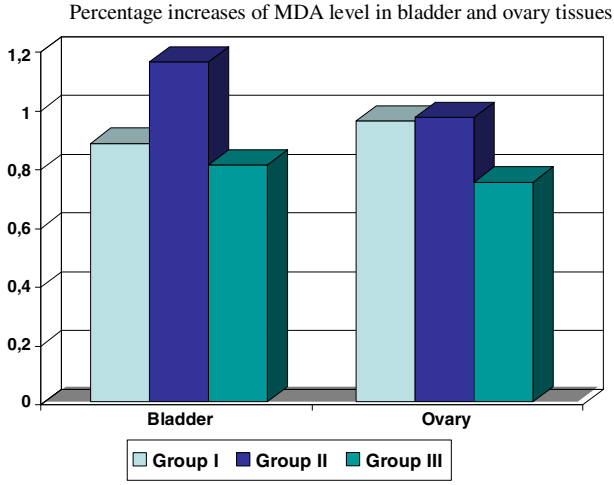
Results

The results are shown in Tables 1 and 2. Contrast medium caused some increases in MDA levels in the liver, bladder, and ovary tissues, and vitamin C prevented it. In the liver tissue, NO level was found to increase in the contrast-treated animals and vitamin C prevented this increase as well. Among the antioxidant parameters measured in the liver tissue, only SOD activity was found to increase in vitamin C-treated group (group II 146.4 ± 25.89 U/mg vs. group III 193.2 ± 28.87 U/mg).

In the histological examination of the tissues, no significant alterations were observed. In Fig. 1, micrographs a and a′ show the regular structure of the liver, which is a solid organ composed of tightly packed hepatocytes. The sinusoids can just be seen as pale-stained spaces between the plates of liver cells. Portal tracts contain the main blood vessels running into the liver. The hepatic lobule is roughly hexagonal in shape and is centered on a terminal hepatic venule. Micrographs b and b′ show the regular structure of the over tissue. In the peripheral zone of the stroma, known as the cortex, are numerous follicles that contain female gametes in various stages of development. Micrographs c and c′ show the regular structure of the urinary bladder with the surface cells called umbrella cells of transitional epithelium lining. Lamina propria is seen underlying the epithelium. The wall of the bladder consists of three loosely arranged layers of smooth muscle and elastic fibers.

Table 2 MDA levels in bladder and ovary tissues (mean ± SD; $n = 7$ in each group)

Groups	Bladder	Ovary
I	0.896 ± 0.205	0.975 ± 0.341
II	1.180 ± 0.803	0.986 ± 0.323
III	0.821 ± 0.348	0.762 ± 0.213



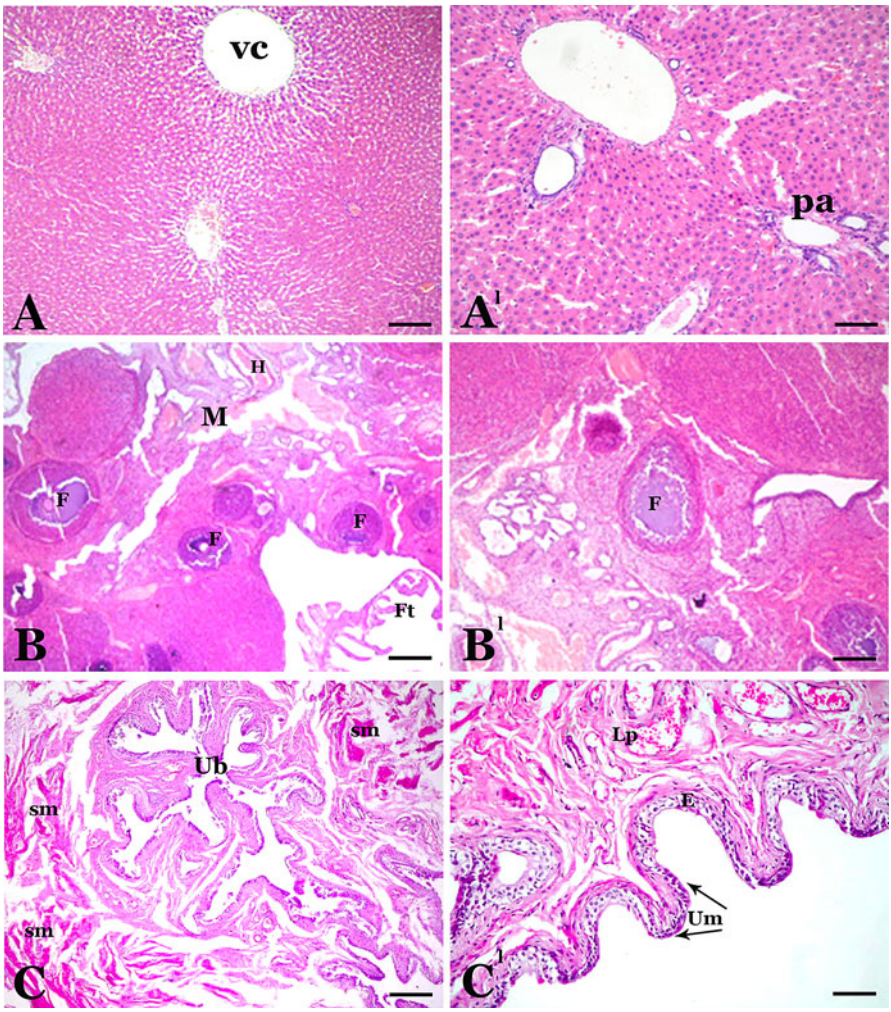


Fig. 1 vc = vena centralis (terminal hepatic venule), pa = portal area, M = medulla, F = follicle, H = helicine artery, Ft = fallopian tube, Ub = urinary bladder, sm = smooth muscle, Lp = lamina propria, E = transitional epithelium, Um = umbrella cells. (**a**, **b**, **b′**, **c**) 80 μm; (**a′**, **c′**) = 40 μm

Discussion

Contrast agents are known to be the major problem with regard to nephrotoxicity (Cetin *et al*., 2008). However, their effects on other organs are not known yet. In fact, no study has investigated metabolic and biochemical changes in the other tissues except kidney tissue associated with contrast infusion. Therefore, as far as we know, this is the first study in this regard. This study was designed to investigate possible effects of ionic high-osmolar contrast medium in liver, bladder, and ovary tissues from rats.

Antioxidants combat with free radicals, thus, preventing oxidative damage in tissues (Gey, 1990; Tardif, 2006). Some studies suggested that consumption of vitamin C-rich foods, such as fruits and vegetables, reduces oxidative damage (Block *et al*., 2001; Joshipura *et al*., 2001; Liu *et al*., 2000). Additionally, some studies have indicated that administration of high-dose vitamin C improves the survival of patients with terminal cancer (Cameron and Campbell, 1974; Cameron and Pauling, 1976, 1978).

Our results show that MDA levels increased in mild degree in the tissues studied from the animals in the contrast-treated group. This finding shows that moderate oxidant stress develops in the tissues due to contrast treatment. It is possible that this effect is dose-dependent, which may be more destructive at the higher exposure times and amounts. We think that increased NO level might contribute to this event because NO exerts powerful oxidative activity under some conditions. It is possible that increased NO or some other oxidative factors due to contrast treatment leads to oxidant stress in the liver, bladder, and ovary tissues, which results in oxidation in the tissues. Fortunately, our results also show that this oxidative increase can be prevented by vitamin C administration in all these tissues. It means that pretreatment with vitamin C may give beneficial effects to lessen this kind of adverse effects of the contrast agents.

Conclusions

Our results suggest that contrast treatment causes mild oxidant stress in the liver, bladder, and ovary tissues, and vitamin C administration can prevent this status. Therefore, we think that vitamin C itself or consuming foods with high vitamin C content before contrast treatment might be beneficial in this respect.

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