Effects of cerium oxide on liver tissue in liver ischemia-reperfusion injury in rats undergoing sevoflurane anesthesia

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Abstract. During liver surgery and transplantation, periods of partial or total vascular occlusion are inevitable and result in ischemia-reperfusion (IR) injury. Nanomedicine uses the latest technological advancement, which has emerged from interdisciplinary efforts involving biomedical sciences, physics and engineering to protect and improve human health. Antioxidant nanoparticles are potential therapeutic agents. The present study investigated the effects of cerium oxide (Co) administration and sevoflurane anesthesia on liver tissue with IR injury. A total of 36 rats were randomly divided into control, Co, IR, IR-Sevoflurane (IRS), Co + IR and Co + IRS groups. In the IR, IRS and Co + IRS groups, hepatic IR was induced. Intraperitoneal Co was administered to the Co groups 30 min before ischemia. Sevoflurane was administered to the IRS and Co + IRS groups during IR injury. Liver tissue samples were examined under the light microscope by staining with hematoxylin and eosin. Thiobarbituric acid (TBARS) levels as well as catalase (CAT) and glutathione-S-transferase (GST) enzyme activity were evaluated in liver tissue samples. The IR group had considerably more hydropic degeneration, sinusoidal dilatation and parenchymal neutrophil infiltration than the Co, IRS, Co + IR and Co + IRS groups. CAT and GST enzyme activity were significantly higher in Co and Co + IR groups compared with the IR group. TBARS levels were significantly lower in Co, IRS, Co + IR and Co + IRS groups compared whit those in the IR group. Intraperitoneal injection of Co with sevoflurane decreased oxidative stress and damage to the liver.

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Introduction

Hepatic ischemia-reperfusion (IR) injury is a biphasic condition defined by a transient interruption in the blood supply to the liver followed by rapid reperfusion (1). Despite developments in liver surgery protocols, IR injury is a concern in hepatic surgery that has an impact on postoperative morbidity and mortality (1). Therefore, it is a concern in hepatobiliary surgery, particularly liver resection and transplantation surgery, in which graft dysfunction is still a problem (1). The best course of action remains a matter of debate.

Due to its intensive metabolic functions, the liver is highly sensitive to redox disturbance. Increased reactive oxygen species (ROS) have been related to a series of molecular events that result in hepatocellular injury (2). Therefore, antioxidants are being used in current research on the treatment of hepatic IR injury to avoid the formation of excessive ROS (3-5).

Cerium oxide (Co) is one of several potent ROS scavengers and its antioxidant effects have piqued attention in the medical industry (6,7). Consequently, Co has been considered a therapeutic agent not only in hepatic IR (8) but also to treat stroke (9), ovarian cancer (10), cardiomyopathy (11), sepsis (12), obesity (13), lower extremity (14), intestinal (15) and lung IR (16).

Volatile agents are an essential part of perioperative medicine and are present in almost every patient undergoing general anesthesia (17). Several anesthetic agents (such as sevoflurane, desflurane, isoflurane, halothane, enflurane and xanthine oxidase) have been shown in various organs of animal models to decrease oxidative damage and inflammation, as well as protect against IR injury (18-20).

Sevoflurane is a halogenated volatile anesthetic that is one of the most commonly used for the induction and maintenance of general anesthesia in all age groups due to its ease of administration, versatility and stable hemodynamic profile (17). Since isoflurane has a recovery time longer than that of sevoflurane and desflurane is more irritating to the airway, sevoflurane induction and recovery tend to be smoother and are associated with fewer complications compared with isoflurane and desflurane (17,21,22).

To the best of our knowledge, although the precise mechanism remains unclear, volatile anesthetics are hypothesized to lessen IR injury and oxidative stress to the liver by lowering inflammatory cytokine levels (23). Sevoflurane inhibited cytokines more effectively compared with other volatiles (23). However, if Co nanoparticles prevent hepatic injury caused by IR under sevoflurane remains unclear. The present study aimed to investigate the combined effects of sevoflurane and Co on IR-injured liver tissue using biochemical and histological examination.

Materials and methods

Animals. Procedures were approved by the Gazi University Ethical Committee of Experimental Animals (approval no. G.Ü.ET-21.064) and performed at the Gazi University Animal Laboratory following the Guidelines for the Care and Use of Laboratory Animals, Ankara, Turkey (24). In the present study, a total of 36 female Wistar albino rats (age, 5 months; weight, 250-350 g), which were supplied by Gazi University Experimental Animals Research Center, were used. Animals were housed under identical environmental conditions and kept in a temperature/humidity controlled room (20-21˚C, 45-55%) under a 12/12-hlight/dark cycle. Food and water were available *ad libitum*.

Experimental groups. A total of 36 rats were randomly assigned and equally (n=6) divided into six groups (Control, Co, IR, IRS, Co + IR and Co + IRS). All surgical procedures were performed under general anesthesia. An intramuscular injection of 50 mg/kg ketamine hydrochloride (500 mg/10 ml; Ketalar®vial; Parke-Davis; Pfizer, Inc.) +10 mg/kg xylazine hydrochloride (Alfazyne® vial 2%; Ege Vet, Ltd.) was administered for anesthesia. After 30 min, the procedure was performed under a warming lamp with the rats in the supine position. In the surgical groups, after skin asepsis was achieved, a midline abdominal incision was applied to the rats and the porta hepatis was explored. In the IR groups, an atraumatic micro clamp was placed on the porta hepatis for 120 min, then the clamp was withdrawn, and the liver was re-perfused for another 120 min. In Co groups 0.5 mg/kg Co was administered intraperitoneally. In all groups, liver tissue of the rats was excised after having been sacrificed under anesthesia and the experiment lasted 270 min in total.

In the control group, rats were anesthetized 30 min before laparotomy. A midline laparotomy was the sole surgical procedure without any additional intervention. Blood samples (5-10 ml) were taken after 4 h follow-up. The liver tissue of rats was excised after having been sacrificed under anesthesia.

In the Co group, 0.5 mg/kg Co (Co aqueous nanoparticle dispersion, 100 ml; Sigma-Aldrich; Merck KGaA) was administered intraperitoneally 30 min before laparotomy. Laparotomy was the sole surgical procedure without hepatic ischemia intervention. At 4 h after the procedure, liver tissue of the rats was excised after having been sacrificed under anesthesia.

In the IR group, hepatic IR was performed following laparotomy. Subsequently, the rats were sacrificed and liver tissue was excised.

In the IRS group, the anesthesia procedure was conducted on the rats in a transparent plastic box. Hepatic IR procedure was performed following laparotomy. During the ischemia period, anesthetic gas vaporizers were calibrated and set at a minimum alveolar concentration of 2.3% sevofluranein oxygen (Sevorane Likid; 250 ml; AbbVie Tıbbi İlaçlar San. ve Tic. Ltd. Şti.).

In the Co + IR group, following laparotomy, Co was administered (0.5 mg/kg) 30 min before the ischemia period and the liver was re-perfused.

In the Co + IRS group, following laparotomy, Co was administered (0.5 mg/kg) intraperitoneally 30 min before ischemia. During the ischemia period, sevoflurane was applied with the 2.3% inspiratory concentration in a transparent plastic box and the liver was re-perfused.

Anesthesia was maintained in the control, Co, IR and Co +IR groups, which did not receive sevoflurane, with injections of 20 mg/kg ketamine with 5 mg/kg xylazine if a positive reaction to surgical stress or intermittent tail pinch was observed. Following the end of the reperfusion period, all rats were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and sacrificed by collecting blood (5-10 ml) from their abdominal aorta. After heartbeat and respiration ceased, rats were monitored for a further 2 min to confirm death. Liver tissue specimens were excised for subsequent biochemical and histopathological analysis.

Histopathological evaluation. Histopathological assessment was performed at the Department of Histology at Kirikkale University. After tissues were fixed in 10% formalin for 48 h at room temperature specimens were prepared with paraffin blocks. Tissue sections of 5-μm stained with hematoxylin for 10 min and then in eosin for 5 min at room temperature. The histopathological assessment and scoring were performed under light microscopy (magnification x100; Nikon Corporation). The same pathologist performed the histological evaluations in a blinded manner.

Each preparation was examined for hepatocyte degeneration, sinusoidal dilatation, pre-necrotic cell and mononuclear cellular infiltration in the parenchyma. Histological testing semiquantitative evaluation technique used by Abdel-Wahhab *et al* (25) was applied for interpreting the structural changes in hepatic tissues of control and treatment groups. According to this, a negative point (0) represents no structural changes; one positive point (1,+) indicates mild changes; two positive points (2,++) represent medium structural changes and three positive points (3,+++) indicate severe structural changes.

Biochemical evaluation. The biochemical examination was performed at the Department of Medical Biochemistry at Gazi University. Oxidative stress and lipid peroxidation in liver tissue was evaluated by measuring thiobarbituric acid (TBA) reactive substance (TBARS) levels and catalase (CAT) and glutathione-S-transferase (GST) enzyme activity.

TBARS assay was performed to measure lipid peroxidation as previously described (26). TBARS assay (CAS Number:122-31-6, Sigma Aldrich, Lot: MKBH2096V) is based on the reaction of malondialdehyde with TBA, which forms a pink pigment with an absorption maximum at 532 nm

Table I. Histopathological data of liver tissue.

Variable	Control (n=6)	Co (n=6)	IR (n=6)	IRS (n=6)	Co + IR (n=6)	Co + IRS (n=6)	Kruskal Wallis P-value	Comparison	P-value
Hydropic degeneration	0.17±0.17[a]	0.33±0.21[a]	1.67±0.33	0.83±0.31[a]	0.50±0.22[a]	0.50±0.22[a]	0.003	Control vs. Co	0.642
								Control vs. IR	<0.0001
								IR vs. Co	0.001
								IR vs. IRS	0.026
								IR vs. Co +IR	0.003
								IR vs. Co +IRS	0.003
Sinusoidal dilation	0.33±0.21[a]	0.50±0.22[a]	1.67±0.33	0.83±0.17[a]	0.50±0.22[a]	0.67±0.21[a]	0.013	Control vs. Co	0.649
								Control vs. IR	0.001
								IR vs. Co	0.003
								IR vs. IRS	0.029
								IR vs. Co +IR	0.003
								IR vs. Co + IRS	0.010
Pycnotic nuclei	0.17±0.17	0.33±0.21	1.00±0.26	0.67±0.21	0.33±0.21	0.50±0.22	0.120	Control vs. Co	1.000
								Control vs. IR	0.154
								IR vs. Co	0.545
								IR vs. IRS	1.000
								IR vs. Co+ IR	1.000
								IR vs. Co +IRS	1.000
Necrosis	0.17±0.17[a]	0.17±0.17[a]	1.33±0.21	0.83±0.21	0.50±0.22[a]	0.67±0.21[a]	0.006	Control vs. Co	1.000
								Control vs. IR	0.001
								IR vs. Co	0.001
								IR vs. IRS	0.118
								IR vs. Co + IR	0.012
								IR vs. Co +IRS	0.040
Parenchymal mononuclear cell infiltration	0.33±0.21[a]	0.50±0.22[a]	1.50±0.22	1.00±0.26	0.50±0.22[a]	0.67±0.21[a]	0.011	Control vs. Co	0.605
								Control vs. IR	0.001
								IR vs. Co	0.004
								IR vs. IRS	0.128
								IR vs. Co +IR	0.004
								IR vs. Co +IRS	0.014

Data are presented as the mean ± standard error. P-values were calculated with Kruskal-Wallis test. [a]P<0.05 vs. IR. Co, cerium oxide; IRS, ischemia-reperfusion-sevoflurane.

in acidic pH and 1,1,3,3-tetraethoxypropane was used as a standard MDA solution freshly in 0.1 M pH 7 TTRIS-HCl buffer solution from concentrated TEP.

CAT activity is based on the measurement of absorbance decrease due to H_2O_2 (Sigma-Aldrich H1009, CAS Number 7722-84-1) consumption at 240 nm as described by Aebi (27).

GST enzyme activity was measured as described by Habig *et al* (28). GST activity method is based on the measurement of absorbance increase at 340 nm due to the monitoring the absorbance increase of the GSH-CDNB complex, which is the product of the GSH (L-Glutathione reduced Sigma-Aldrich G4251, CAS Number 70-18-8) and CDNB (1-Chloro-2,4-dinitrobenzene Sigma-Aldrich 138630 CAS Number 97-00-7) reaction. The results were expressed in IU/mg protein for CAT and GST, nmol/mg protein for TBARS.

Statistical analysis. SPSS 20.0(IBM Corp.) was used for statistical analysis. Data were analyzed using Kruskal-Wallis test followed by Dunn's test or one-way ANOVA followed by post hoc Tukey's test. P<0.05 was considered to indicate a statistically significant difference. Data are expressed as the mean ± standard error.

Results

Histopathological results. Hydropic degeneration, sinusoidal dilation, necrosis and parenchymal mononuclear cell infiltration were significantly different between the groups (hydropic degeneration; P=0.003, sinusoidal dilation; P=0.013, necrosis; P=0.006 and parenchymal mononuclear cell infiltration; P=0.011; Table I).

In comparison with the control group (Fig. 1), hydropic degeneration was more common in IR (P<0.0001; Fig. 2).

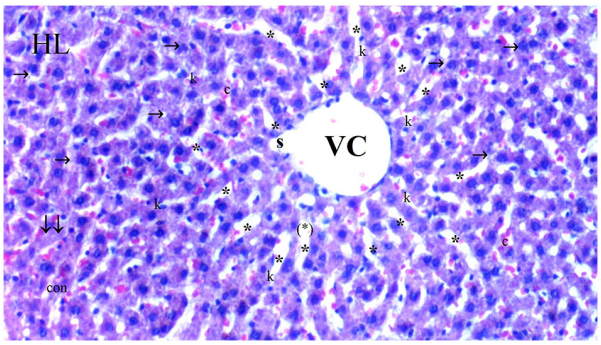

Figure 1. Representative light microscopy of hepatic tissue from the control group. Normal liver tissue. Magnification, x100. HL, hepatic lobule; VC, vena centralis; k, Kupffer cell hyperplasia; *, sinusoid dilatation; ↓↓, infiltration; →, hepatocyte; c, dikaryotic hepatocyte; con, congestion.

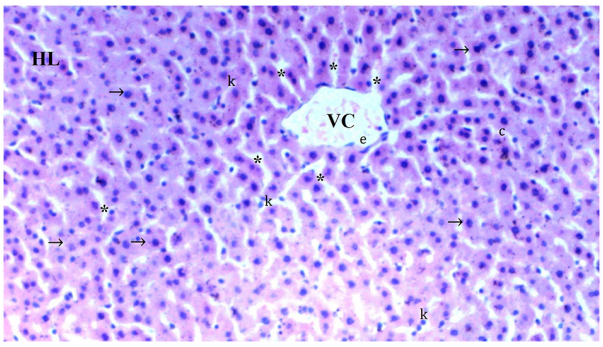

Figure 2. Representative light microscopy of hepatic tissue from the ischemia-reperfusion group. HL, hepatic lobule; VC, vena centralis; e, erythrocyte; *, sinusoid dilatation; →, hepatocyte; c, dikaryotic hepatocyte. H&Ex100.

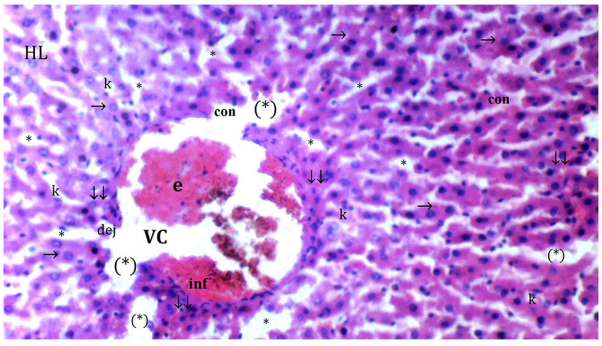

Figure 3. Representative light microscopy of hepatic tissue from cerium oxide group. HL, hepatic lobules; VC, vena centralis; e, erythrocyte; con, congestion; *, sinusoid dilatation; →, hepatocyte; c, dikaryotic hepatocytes; (*), necrotic and apoptotic hepatocyte; ↓↓, infiltration; dej, hydrophilic degeneration; inf, inflammation; k, Kupffer cell hyperplasia. H&Ex100.

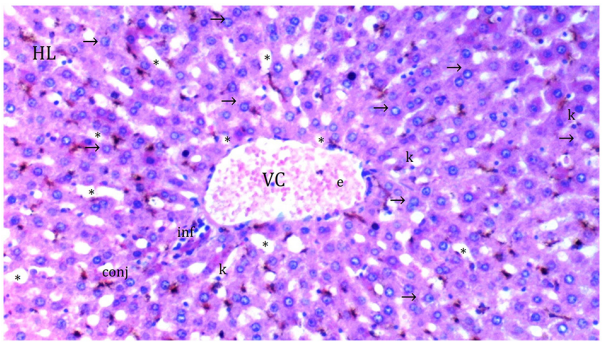

Figure 4. Representative light microscopy of hepatic tissue from ischemia reperfusion-sevoflurane group. HL, hepatic lobule; VC, vena centralis; e, erythrocyte; conj, congestion; *, sinusoid dilatation; inf, inflammation; →, hepatocyte; k, Kupffer cell hyperplasia. H&Ex100.

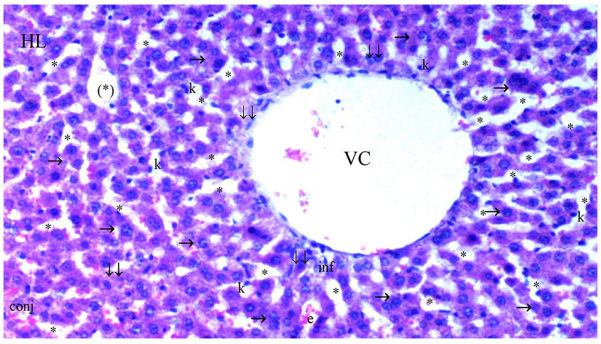

Figure 5. Representative light microscopy of hepatic tissue from cerium oxide-ischemia reperfusion group. HL, hepatic lobule; VC, vena centralis; con, congestion; *, sinusoid dilatation; ↓↓, infiltration; →, hepatocyte; c, dikaryotic hepatocyte; k, Kupffer cell hyperplasia; inf, inflammation; conj, congestion; (*), necrotic and apoptotic hepatocyte. H&Ex100.

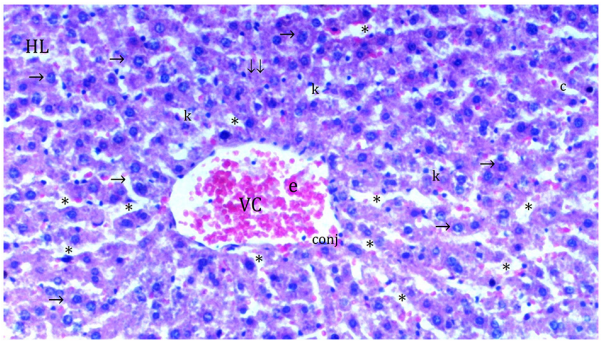

Figure 6. Representative light microscopy of hepatic tissue from cerium oxide-ischemia reperfusion-sevoflurane group. HL, hepatic lobule; VC, vena centralis; e, erythrocyte; conj, congestion; *, sinusoid dilatation; ↓↓, infiltration; →, hepatocyte; c, dikaryotic hepatocyte; k, Kupffer cell hyperplasia. H&Ex100.

Hydropic degeneration was found to be significantly lower in Co (Fig. 3), IRS (Fig. 4), Co + IR (Fig. 5) and Co + IRS (Fig. 6) groups compared with those in the IR group (P=0.001, P=0.026, P=0.003 and P=0.003, respectively; Table I).

Sinusoidal dilatation was more common in the IR than in the control group (P=0.001). Sinusoidal dilatation was significantly lower in the Co (Fig. 3), IRS (Fig. 4), Co + IR (Fig. 5) and Co + IRS (Fig. 6) groups compared with the IR group (P=0.003, P=0.010, P=0.003 and P=0.029, respectively; Table I).

Necrosis was more common in the IR group than in the control group (P=0.001; Table I). Necrosis was significantly lower in Co (Fig. 3), Co + IR (Fig. 5) and Co + IRS (Fig. 6) groups than in the IR group (P=0.001, P=0.012, P=0.040,

Table II. Biochemical data of liver tissue.

Variable	Control (n=6)	Co (n=6)	IR (n=6)	IRS (n=6)	Co + IR (n=6)	Co + IRS (n=6)	ANOVA P-Value	Comparison	P-value
Thiobarbituric acid, nmol/ml	0.36±0.04[a]	0.45±0.05[a]	1.22±0.24	0.55±0.07[a]	0.44±0.05[a]	0.47±0.04[a]	0.0001	Control vs. Co	0.566
								Control vs. IR	<0.0001
								IR vs. Co	<0.0001
								IR vs. IRS	<0.0001
								IR vs. Co + IR	<0.0001
								IR vs. Co + IRS	<0.0001
Catalase, IU/mgpro	2.69±0.37[a]	2.51±0.40[a]	0.93±0.23	1.67±0.36	2.60±0.50[a]	1.93±0.35[a]	0.016	Control vs. Co	0.737
								Control vs. IR	0.002
								IR vs. Co	0.006
								IR vs. IRS	0.172
								IR vs. Co +IR	0.004
								IR vs. Co + IRS	0.043
Glutathione-S-transferase, IU/mgpro	0.62±0.20[a]	0.49±0.15[a]	0.05±0.01	0.26±0.11	0.53±0.17[a]	0.42±0.12	0.049	Control vs. Co	0.523
								Control vs. IR	0.007
								IR vs. Co	0.030
								IR vs. IRS	0.296
								IR vs. Co +IR	0.019
								IR vs. Co +IRS	0.068

Data are presented as the mean ± standard error. P-values calculated with ANOVA. [a]P<0.05 vs. IR. Co, cerium oxide; IRS, ischemia-reperfusion-sevoflurane; pro, protein.

respectively; Table I) while it was similar between IR and IRS groups (Fig. 6; P=0.118; Table I).

Parenchymal mononuclear cell infiltration was significantly decreased in the Co, Co + IR and Co + IRS groups compared with that in the IR group (P=0.004, P=0.004 and P=0.014, respectively) while it was found similar between IR and IRS groups (P=0.128; Table I).

The number of pyknotic nuclei was similar between all groups (P=0.120; Table I).

Biochemical results

TBARS levels. A significant difference was found in levels of TBARS in the liver tissue between groups (P<0.0001; Table II). In the IR group, TBARS levels were higher than in the control group (P=0.001). TBARS levels were significantly lower in Co, IRS, Co + IR and Co + IRS groups compared with in the IR group (P<0.0001 for all; Table II).

CAT enzyme activity. CAT enzyme activity in liver tissue was significantly different between all the groups (P=0.016; Table II).CAT enzyme activity was found to be significantly decreased in the IR group compared with that in the control group (P=0.002; Table II). CAT enzyme activity in Co, Co + IR and Co + IRS groups was significantly increased compared with that in the IR group (P=0.006, P=0.004, P=0.043, respectively; Table II). There was no difference between IR and IRS groups (P=0.172; Table II).

GST enzyme activity. GST enzyme activity in liver tissue was significantly different between the groups (P=0.049; Table II). In the IR group, GST enzyme activity was significantly lower than that in the control group (P=0.007) and increased compared with that in the Co and Co + IR groups (P=0.030 and P=0.019, respectively; Table II). There was no difference between IR and IRS and Co + IRS groups (P=0.296 and P=0.068, respectively; Table II).

Discussion

Hepatic IR is a severe issue that impairs graft function, particularly in liver transplantation (1,29). It involves a short halt in blood flow to all or part of the liver, followed by rapid reperfusion, disrupting normal homeostatic systems and generating free radicals (1). Hepatocellular injury and mortality are associated with high levels of ROS and the consequent activation of an inflammatory cascade (2). Certain approaches, such as Co and inhalation anesthetics, may decrease the severity of IR-induced harm (8,16). Co proven to be beneficial in fatty liver, fibrosis and drug-induced hepatocidal toxicity, such as doxorubicin and paracetamol, in addition to IR models (6,30-32). The present study investigated the protective effect of Co in a rat model of experimental hepatic IR damage. To the best of our knowledge, this is the first study to combine Co with sevoflurane in a liver IR model.

Several enzymes protect cells from IR-induced oxidative damage by acting as intracellular antioxidants. While the present study did not directly measure ROS levels, it investigated TBARS levels and CAT and GST enzyme activities, as well as histological examination using H&E staining to see if Co had a therapeutic impact. The TBARS assay, which detects MDA, is a common laboratory test for determining the degree

of harm caused by free radicals generated by IR (33). Cellular antioxidant defense functions are supported by CAT and GST. These enzymes degrade superoxide anions and hydrogen peroxide while also preventing the formation of free radicals. Antioxidant efficacy is shown by high levels of CAT and GST in the blood (34). In the present study, increased TBARS levels were present in the IR group and CAT and GST enzyme activities revealed the protective effect of Co on liver IR. In the hepatic tissue of rats that received Co before hepatic IR, there was a considerable decrease in TBARS levels, as well as a significant rise in CAT and GST enzyme activity compared with those in the IR group; this finding was consistent with earlier investigations (6,8).

The hepatoprotective effects of Co were confirmed by histological findings. The IR damage was linked with notable hepatocyte degradation, sinusoidal dilatation, parenchymal mononuclear infiltration and several regions of necrosis, according to histological examination. Treatment with CO_2 h before hepatic IR prevented these alterations and protected hepatocellular architecture. These modifications showed that Co can reduce ROS-induced cell death and thereby protect hepatocytes from IR-induced damage. This may be attributed to excess caspase 3 and inflammatory cytokine levels as well as reduced macrophage infiltration in presence of Co (32).

Although most antioxidants used to treat liver disease have difficulty targeting hepatocytes, necessitating repeated administration at high concentrations (35), in the present study Co was administered once before ischemia. Yokel *et al* (36) revealed that Co nanoparticles exist in the circulation for a short time on intravenous administration (half-life, 7.5 min).Even if the remaining intravenous time is limited, nanoparticles translocate to the liver and other organs (37). Nanoceria, nanoparticles of cerium oxide, in particular, has been demonstrated to be taken up by Kupffer cells in the liver, in which nanoceria partially dissolves to generate second-generation nanoceria clouds, which are smaller and may be more effective at reducing free radicals (38). Manne *et al* (8) demonstrated that Co nanoparticles protect against hepatic IR injury by infusing 0.5 mg/kg of 10-30 nm spherical Co nanoparticles intravenously into Sprague-Dawley rats 1 h before inducing hepatic ischemia in the left lateral and median lobes. The present study administered 0.5 mg/kg Co intraperitoneally for 2 h before the ischemia. It was speculated that a single intraperitoneal dose of Co may preserve liver tissue in IR models in rats without the requirement for numerous intravenous administrations due to *in vivo* distribution and cellular uptake of nanoceria and intrinsic autocatalytic activity.

Sevoflurane, desflurane and isoflurane, all extensively used volatile anesthetics in clinical practice, may be viable options for reducing IR damage. Through the control of inflammatory cytokines, oxidative stress and complement, they protect against hepatic IR damage (19,23,39). However, compared with isoflurane, sevoflurane has a stronger effect in reversing liver function, inhibiting inflammatory cytokines and decreasing oxidative stress (23). There is interest in the non-anesthetic effects of sevoflurane. Its principal mechanisms are lowering oxygen free radical and excess calcium level, suppressing inflammatory responses and enhancing liver cell energy consumption (40). Both experimental and clinical investigations imply that the mechanism of sevoflurane conditioning in decreasing hepatic IR damage is similar to that of ischemia preconditioning (39,41,42).

Although it remains unclear how sevoflurane reduces hepatic IR, some mechanisms have been discussed (43). Sevoflurane attenuates aggregation of macrophages and neutrophils in the liver sinusoid. Furthermore, it preserves the endothelial glycocalyx, reduces apoptosis and exerts antioxidant effects by regulating the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, thereby decreasing liver IR injury (44). The transcription factor Nrf2 is a pivotal agent in protection against oxidative stress. It is involved in the regulation of the expression of antioxidants, such as GST (45). In the absence of Nrf2, liver regeneration is significantly delayed and hepatocyte death is also increased (46). It has been demonstrated that Co and sevoflurane both downregulate Nrf2 (44,46).

The protective effect of sevoflurane in liver IR injury was revealed by the present investigation. When compared with the IR group, the IRS group had lower levels of TBARS, hepatocyte degeneration and sinusoidal dilatation. The combination of sevoflurane and Co also decreased IR damage in the liver. The significant decrease in TBARS levels shows that damage was associated with lipid peroxidation. Co and sevoflurane administration may decrease IR damage by altering lipid peroxidation. Histopathological findings supported the biochemical findings, demonstrating that co-administration of Co with sevoflurane may be more effective in avoiding liver damage than sevoflurane alone. Compared with the IR group, the Co + IR group exhibited notably decreased hepatocyte deterioration, sinusoidal dilatation, parenchymal mononuclear infiltration and necrosis.

The primary limitation of the present study was the absence of aspartate transaminase (AST) and alanine transaminase (ALT) level measurements. ALT and AST are standard biomarkers of choice for detecting liver injury (47). However, considering ALT and AST are not liver-specific and limited blood volume in rats, CAT, TBARS and GST were selected. ALT levels should be reported in future studies to improve the understanding of the effect of Co on liver tissue in the IR model.

In summary, the present findings revealed that Co therapy decreased oxidative stress generated by IR, as evidenced by a decrease in TBARS levels and increased activity of CAT and GST. The biochemical and histological findings in rats reveal a decrease in liver damage in Co + IR and Co +IRS groups compared with IR group. These findings support the hepatoprotective effects of Co. Taken together, the findings imply that intraperitoneal 0.5 mg/kg prophylactic Co administration may be a potential therapeutic method for treatment of hepatic IR damage. These effects should be confirmed at different concentrations and dosing regimens.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MA and AK designed the study and analyzed and interpreted data. HG, CO and EK performed the experiments. SE and MA analyzed and interpreted data. MA, SE and HG confirm the authenticity of all the raw data. XX, AK, MA and MK provided scientific and technical assistance and critically revised the article for important intellectual content. CO and MA collected samples. TM and MK performed cellular and molecular experiments. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval for the study was obtained from Gazi University Experimental Animals Ethics Committee (Ankara, Turkey; approval no:G.Ü.ET-21.064).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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