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Attenuation of intestinal ischemia/reperfusion induced liver and lung injury by intraperitoneal administration of (−)-Epigallocatechin-3-gallate

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Abstract

The aim of this study was to evaluate the effect of (−)-epigallocatechin-3-gallate (EGCG), a natural antioxidant, on liver and lungs after warm intestinal ischemia/reperfusion (I/R). Thirty male Wistar rats were equally divided into a sham-operation group, an intestinal I/R group and an intestinal I/R group pretreated with EGCG intraperitoneally. Intestinal ischemia was induced by occlusion of the superior mesenteric artery for 60 min followed by reperfusion for 120 min. Immediately after reperfusion, liver, lung and blood samples were collected and analyzed. Results showed that intestinal I/R increased ($p < 0.05$) the levels of aspartate (AST) and alanine (ALT) transaminase in serum to 987 and 752 IU/l, respectively. Malondialdehyde (MDA) increased ($p < 0.05$) in liver to 1.524 nmol/g in the group subjected to intestinal I/R compared to 0.995 nmol/g in the sham operation group. MDA was also increased ($p < 0.05$) in lungs to 1.581 nmol/g compared to 0.896 nmol/g in the sham operation group. Myeloperoxidase (MPO) increased ($p < 0.05$) in liver, after intestinal I/R, to 5.16 U/g compared to 1.59 U/g in the sham operation group. MPO was also increased ($p < 0.05$) in lungs to 3.89 U/g compared to 1.65 U/g in the sham operation group. Pretreatment with EGCG decreased ($p < 0.05$) serum levels of AST and ALT to 236 and 178 IU/l, respectively. It also decreased ($p < 0.05$) mean MDA levels in liver and lungs to 1.061 and 1.008 nmol/g, respectively, and mean MPO levels in liver and lungs to 1.88 and 1.71 U/g, respectively. Light microscopy and transmission electron microscopy examinations showed significant alteration in liver and lungs and protection of liver and lung parenchyma in the animals treated with EGCG.

Keywords: *Intestinal ischemia/reperfusion, liver, lungs, epigallocatechin-3-gallate, antioxidants, lipid peroxidation*

Introduction

Intestinal ischemia/reperfusion (I/R) injury is considered as a major clinical problem and may occur in patients suffering from coagulopathies, mechanical obstruction, severe trauma, patients undergoing aortic or cardiac surgery and also intestinal transplantation surgery [1]. It is associated with hemorrhage and shock and characterized by microvascular and mucosal alterations including endothelial cell swelling, capillary plugging and prolonged reduction in intestinal blood flow, mucosal barrier impairment and also polymorphonuclear leucocyte (PMN) infiltration [2–6]. Gut barrier

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dysfunction holds an important part on the pathogenesis of systemic inflammatory response syndrome upregulating the expression of adhesion molecules on the surface of endothelial membrane and the production of inflammatory mediators [5–7]. The increased endothelial permeability results in a tissue edema, a PMN recruitment, and increased portal and systemic plasma endotoxin levels,which may result in activation of gut macrophages and Kupffer cells (KCs) to produce inflammatory mediators [8–12].

Remote organs are affected by gut I/R, including the liver and the lung, which appear to be the most vulnerable [8]. Intestinal I/R provokes hepatocellular injury and liver dysfunction, as well as an acute lung injury characterized by an increased pulmonary vascular permeability and a non cardiogenic pulmonary edema, leading to adult respiratory distress syndrome and finally to a systemic inflammatory response syndrome [1,8,13–14].

The intestinal mucosa is very susceptible/sensitive to ischemia. Primarily, gut ischemia and the consequent bacterial translocation and systemic endotoxin release triggers a production of inflammatory mediators, adhesion molecules and reactive oxygen species (ROS), which is followed by activation and accumulation of PMN [5–7,15–16]. Neutrophils are considered as main mediators of the hepatocellular alterations regarding gut I/R [8]. Activation of Kupffer cells, the resident macrophages of the liver, is considered as a major contributor to PMN aggregation, impaired sinusoidal perfusion and liver tissue hypoxia following gut I/R [1,8,9].

(−)-Epigallocatechin-3-gallate (EGCG) is the most active polyphenol of green tea [17,18]. Green tea catechins have been reported to display potent antioxidant, antiviral, antibacterial, hypocholesterolemic and anticarcinogenic properties [18–23]. EGCG has been proven effective in preventing copper-induced oxidation of LDL *in vitro* [3], attenuation of lipid peroxidation and inhibition of xanthine/oxidase activity [17]. It also shows high efficiency in scavenging ROS and in inhibiting inducible- and neuronal- nitric oxide synthase activity and gene expression [17,21,24,25]. EGCG is the most abundant component of green tea catechins and is considered to have stronger physiological activities compared to other catechins [3,17,18,23].

The aim of this study was to evaluate the effect of EGCG on liver and lung injury after warm intestinal I/R.

Materials and methods

Thirty male Wistar rats weighing 250–300 g were used in this study. The rats, cared for in accordance with the Guide for the Care and Use of Laboratory Animals [26], were kept under a 12-h light–dark cycle and permitted *ad libitum* access to standard laboratory rodent chow and tap water for 2 weeks before the beginning of the experimental procedure.

The animals were divided into three groups. One group was subjected to sham operation. The second was subjected to intestinal I/R and served as the control receiving the same amount of 0.9% normal saline as a vehicle. The third group was subjected to intestinal I/R with intraperitoneal administration of EGCG (Hunan Kinglong Bio-resource Co. Ltd, Hunan, China) 15 min prior to ischemia at a dose of 50 mg/kg body weight.

In the I/R groups, a midline laparotomy was performed, and heparin (Heparin® 5000, Astra Pharmaceuticals, Sweden) was administered intravenously at 50 IU/Kg to prevent thrombosis of the superior mesenteric artery due to clamping. Intestinal ischemia was induced for 60 min by clamping the superior mesenteric artery with a vascular microclip (Scanlan® International, St Paul, Minnesota, USA). Removal of the microclip allowed intestinal blood reflow. Following 120 min of reperfusion, the liver and lungs were surgically removed for malondialdehyde (MDA) and myeloperoxidase (MPO) assays, light histology and transmission electron microscope examinations. At the end of reperfusion, blood samples were also obtained by cardiac puncture for determination of aspartate (AST) and alanine (ALT) transferases.

In the sham operation group, the abdomen was opened, heparin (Astra, Stockholm, Sweden) was administered, and the liver and lungs were surgically removed for MDA and MPO assay, light microscopy and transmission electron microscope examinations. Blood samples were obtained by cardiac puncture for AST and ALT assays. Blood samples from all groups were centrifuged at 1200*g* for 10 min, and the serum was obtained and stored at −40°C pending analysis.

Determination of MDA in liver and lung tissue

Determination of MDA, the compound used as an index of lipid peroxidation, was carried out with a selective third-order derivative method [27]. In brief, 1-g samples were thoroughly homogenized (Polytron homogenizer, PCU, Switzerland) with 5 ml of 5% aqueous trichloroacetic acid (Merck, Germany), and 2 ml of 0.8% butylated hydroxytoluene (Sigma Chemical Co., St Louis, MO, USA) in hexane (Merck) were added and centrifuged. The top layer was discarded, and a 2.5-ml aliquot from the bottom layer was mixed with 1.5 ml of 0.8% aqueous 2-thiobarbituric acid (Sigma Chemical Co.) to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled to room temperature and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, Japan) in the range 400–650 nm with a scanning speed of 480 nm/min. Third-order derivative spectra were

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obtained by electronic differentiation (derivative difference setting, 21 nm) of the conventional absorption spectra of samples from both control and drug-treated rats. MDA concentration (nmol/g wet tissue) was calculated on the basis of the third-order derivative peak height at 532 nm by referring to slope and intercept data of the computed least-squares fit of standard calibration curve.

Determination of myeloperoxidase in liver and lung tissue

Tissue-associated MPO activity was determined by a modification of the method of Krawisz et al. [28–29]. Briefly, 1-g samples were homogenized (Polytron homogenizer, PCU, Switzerland) in 10 ml of ice-cold 0.02 M ethylenediaminotetraacetic acid (EDTA), pH 4.7, for 60 s. A 5-ml portion of the tissue homogenate was centrifuged at 20,000*g* for 15 min at 4°C, and the supernatant, which contained $< 5\%$ of the total MPO activity, was discarded. The pellet was homogenized in 5 ml of potassium phosphate buffer, pH 5.4, containing 0.5% hexadecyltrimethylammonium bromide, centrifuged at 20,000*g* for 15 min at 4°C, and the supernatant was collected. A 100-μl portion of the supernatant was mixed with 100 μl of 1.6 mM tetramethylbenzidine in 8% *N*,*N*-dimethylformamide, 30 μl of 0.3 mM H_2O_2 and 770 μl of 80 mM potassium phosphate buffer (pH 5.4) to reach a total volume of 1000 μl. The mixture was incubated at 37°C for 3 min, cooled in an ice bath, and the MPO activity was assessed spectrophotometrically (Shimadzu, Model UV-160A) by measuring the H_2O_2-dependent oxidation of tetramethylbenzidine at 655 nm [30]. One unit of enzyme activity was defined as the amount of MPO causing an absorbance change of 1.0/min at a wavelength of 655 nm at 25°C.

Determination of serum AST and ALT

To assess the damage induced by intestinal I/R to hepatic parenchyma, serum AST and ALT levels were determined by using a Technicon RA-1000 autoanalyser.

Light microscopy examination

Double blind analysis was applied on all liver and lung samples. For the light microscopy process, the specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, cut in sections of 5 μm thickness and stained with hematoxylin-eosin.

Transmission electron microscopy

For transmission electron microscopy, the specimens were immediately immersed in 2.3% glutaraldehyde in phosphate buffered saline (PBS), pH 7.4, for 3 h, washed in PBS, and post fixed in 2% aqueous osmium tetroxide for 1.5 h. The samples were dehydrated through ascending grades of ethanol, infiltrated and embedded in Epon 812. Ultra thin sections were prepared using an ultra cut Reichert-Jung microtome. The sections were stained with aqueous solutions of uranyl acetate and lead citrate, and examined under Jeol 2000 CX electron microscope.

Statistical analysis

Overall significance after ischemia and reperfusion was tested by one-way analysis of variance. The homogeneity of the variances was tested using the Levene's test. Differences between means were tested for significance by Duncan's multiple range test [31]. Statistical analyses were performed using the general linear model of SPSS statistical package (SPSS 10.05, SPSS Ltd., Woking Surrey, UK).

Results

MDA in liver tissue

The animals subjected to intestinal I/R displayed increased ($p < 0.05$) the MDA levels in liver compared to the sham operation animals. In the latter, the mean MDA level was 0.995 ± 0.12 nmol/g wet tissue, whereas in the I/R animals given normal saline mean MDA was 1.524 ± 0.16 nmol/g wet tissue. Administration of EGCG to the animals prior to I/R resulted in marked reduction ($p < 0.05$) of the mean MDA level to 1.061 ± 0.11 nmol/g wet tissue (Figure 1).

MDA in lung tissue

The mean MDA level in lung tissue of the sham operation animals was 0.896 ± 0.15 nmol/g wet tissue. The animals subjected to intestinal I/R, displayed increase ($p < 0.05$) of the mean MDA level to 1.581 ± 0.21 nmol/g wet tissue, whereas the animals pretreated with EGCG showed marked

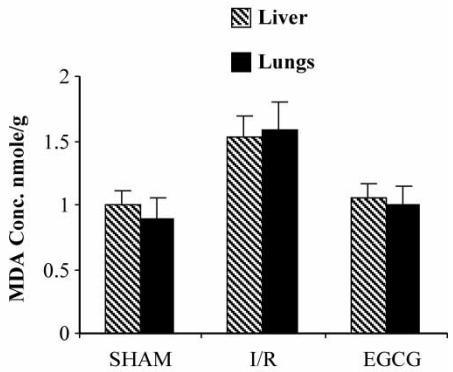

Figure 1. Mean concentrations of MDA in liver and lungs in the sham operation group (SHAM), I/R group (I/R) and I/R group treated with EGCG (EGCG). Values are means of 10 samples ± SD.

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reduction ($p < 0.05$) of the mean MDA level to 1.008 ± 0.13 nmol/g wet tissue (Figure 1).

MPO in liver tissue

The animals subjected to intestinal I/R displayed increased ($p < 0.05$) mean MPO levels in liver compared to the sham operation animals. In the latter, the mean MPO level was 1.59 ± 0.29 U/g, whereas in the I/R animals given normal saline it was 5.16 ± 0.76 U/g. Administration of EGCG to animals prior to I/R, resulted in marked reduction ($p < 0.05$) of the mean MPO level to 1.88 ± 0.38 U/g (Figure 2).

MPO in lung tissue

Mean MPO level in lungs of the sham operation animals was found to be 1.65 ± 0.23 U/g. The animals subjected to intestinal I/R, displayed increase ($p < 0.05$) of the mean MPO level to 3.89 ± 0.21 U/g, whereas the animals pretreated with EGCG showed reduction of ($p < 0.05$) to 1.71 ± 0.35 U/g (Figure 2).

AST and ALT levels in serum

Mean AST values in serum of the sham operation, I/R and EGCG groups were 78 ± 12, 987 ± 29 and 236 ± 31 I/Ul, respectively. Mean ALT values in serum of the sham operation, I/R and EGCG groups were 62 ± 12, 752 ± 27 and 178 ± 19 IU/l, respectively (Figure 3).

Light microscopy examination in liver

In the animals subjected to intestinal I/R, the hepatocytes were presented with cytoplasmic edema, which was prominent in the area around the nucleus. There was a gradual transformation of the nucleus according to the severity of the cytoplasmic edema. Where there was limited cytoplasmic edema, only the marginated heterochromatin was very condensed. In the presence of severe edema, the whole nucleus appeared condensed and accompanied by hepatocyte destruction. The sinusoids were dilated and a number of blood cells were concentrated in the lumen. The

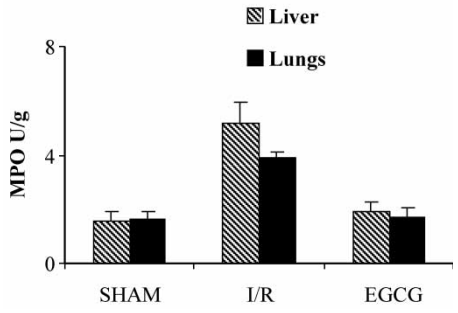

Figure 2. Mean concentrations of MPO in liver and lungs in the sham operation group (SHAM), I/R group (I/R) and I/R group treated with EGCG (EGCG). Values are means of 10 samples ± SD.

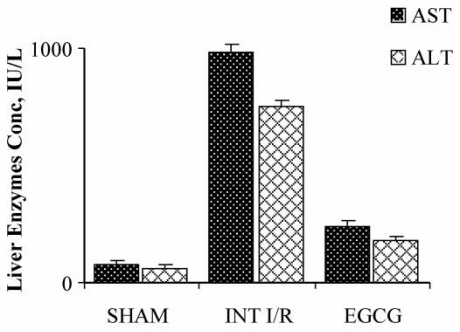

Figure 3. Mean concentrations of AST and ALT in serum of the sham operation group (SHAM), I/R group (I/R) and I/R group treated with EGCG (EGCG). Values are means of 10 samples ± SD.

nuclei of Kupffer cells or endothelial cells in the majority appeared condensed (Figure 4a).

Treatment with EGCG eliminated or disappeared the cytoplasmic edema of hepatocytes, whereas few cells showed condensed marginated heterochromatin and very rarely nucleus condensation. The cytoplasm of the hepatocytes was granular and full of organelles. Few Kupffer and endothelial cells showed condensed nuclei. The sinusoids usually were appeared normal and no blood cells were observed accumulated in the lumen (Figure 4b).

In sham operated animals, hepatocytes were organized in plates directed to the central vein and separated by the sinusoids. They were polyhedral with differences in size and shape even in the adjacent cells and contained one or more, large round, usually centrally located nuclei. The nuclei contained one or more nucleoli and scattered clumps of chromatin. The cytoplasm was granular and full of organelles. Lipid droplets or vacuolization in the cytoplasm were absent (Figure 4c).

Light microscopy examination in lungs

In lungs, a great number of inflammatory cells infiltrated the interstitium, congested the alveoli and increased the thickness of septals. The majority of the inflammatory cells were polymorphonuclear neutrophils. The space of the alveoli was very restricted (Figure 5a).

After treatment, the interstitium of lungs appeared thinner and the number of inflammatory cells decreased. The alveoli were larger in comparison to the non-treated animals and their wall was thinner (Figure 5b).

In sham operated animals, the wall of the respiratory bronchioles and their subdivisions appeared to be very thin (Figure 5c).

Transmission electron microscopy in liver

Ultrastructural examination of liver cells after I/R of the intestine showed severe damage of the mitochondria, dilated smooth endoplasmic reticulum and decreased rough endoplasmic reticulum. The

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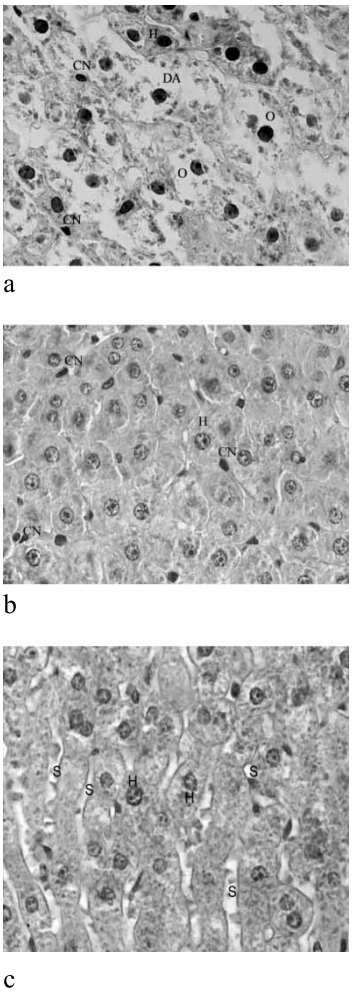

Figure 4. Light microscope photograph of liver. In the control I/R group (a), few hepatocytes appear normal (H), most of them are damaged (DA), and severe edema (O) occupies the area between them. The nucleus of some Kupffer or endothelial cells is condensed (CN) (× 400). In the EGCG group (b), most hepatocytes (H) appear normal. Few endothelial or Kupffer cells contain condense nuclei (CN) (× 400). In the sham operation group (4c) there was normal appearance of hepatocytes (H) and sinusoids (S). (× 400).

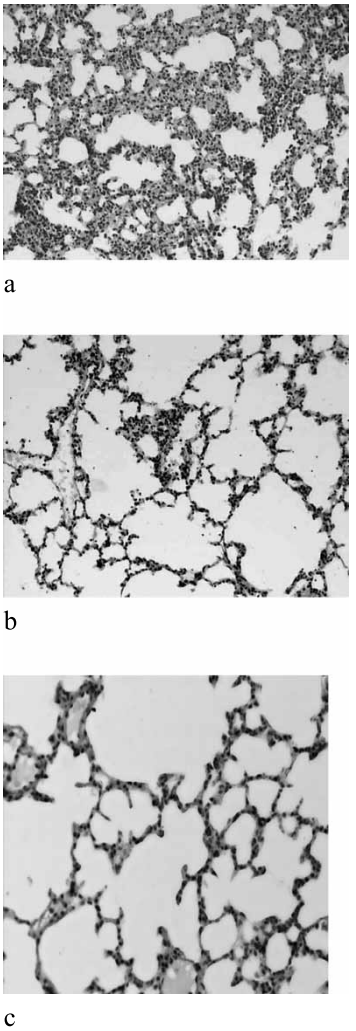

Figure 5. Light microscope photograph of lungs. In the control I/R group (a) a great increase of the interstitium is observed (× 100). In the EGCG group (b) the interstitium is decreased and the alveoli are expanded (× 100). In the sham operation group (5c) there was normal appearance of bronchioles from a respiratory zone × 160.

mitochondria appeared swollen, with edema in the matrix and disorganized cristae. In some of them, the matrix was totally disappeared (Figure 6a).

After treatment, the mitochondria appeared normal, the arrangement of the cristae in the matrix was totally recovered and there was no edema observed in the matrix. Cisternae of rough endoplasmic reticulum were arranged between or around the mitochondria. The smooth endoplasmic reticulum was eliminated and glycogen depositions were scattered throughout the smooth endoplasmic reticulum membranes (Figure 6b).

Transmission electron microscopy in lungs

Ultrastructural examination of lungs after intestinal I/R showed severe narrowing of the alveoli and the presence of debris from destructed epithelial cells into the lumen. The debris contained disorganized mitochondria in different stages, parts from protrusions of squamous epithelial cells, small vesicles and tubular myelin between them (Figure 7a). The mitochondria of the great alveolar cells appeared degenerated. The cristae were disoriented and vesicle formations appeared in the matrix, which was eliminated significantly (Figure 7b).

After treatment, the alveoli were expanded and the lumen was clean and free of cytoplasmic elements. The great alveolar cells appeared normal, the mitochondria were smaller and the cristae and matrix were distributed equally. The squamus epithelial cells were thin and no protrusions of the cell membrane were observed (Figure 7c).

Discussion

Intestinal ischemia may lead to injury of remote organs, including liver and lungs [8], by triggering activation of resident macrophages and KCs that

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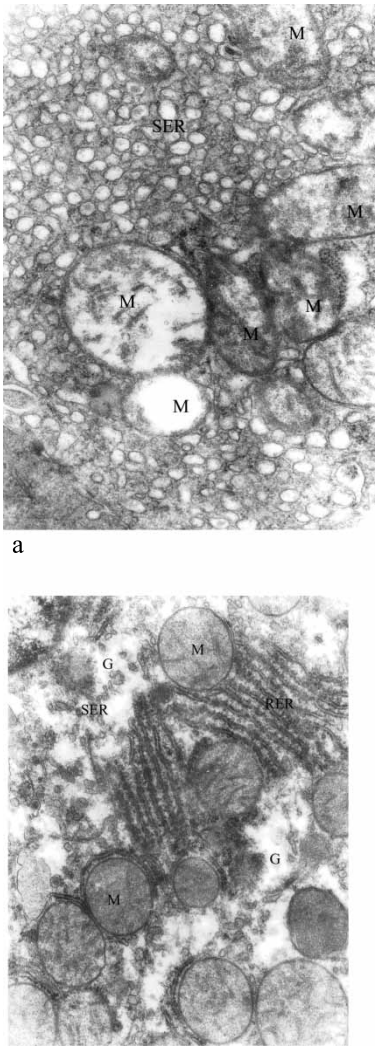

Figure 6. Transmission electron micrograph of liver in the control I/R group (a) the mitochondria are disorganized (M) and the smooth endoplasmic reticulum is dilated (SER) (× 15,000). In the EGCG group (b) the mitochondria are normal (M), the rough endoplasmic reticulum (RER) is arranged between or around them, the smooth endoplasmic reticulum (SER) is decreased and glycogen particles (G) are deposited throughout the cytoplasm (× 12,000).

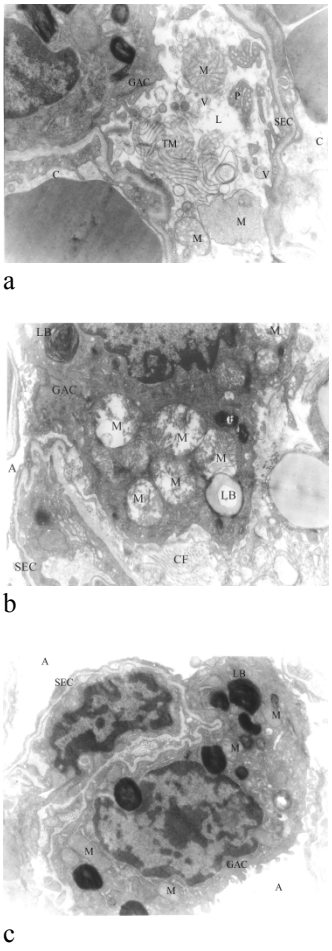

Figure 7. Transmission electron micrograph of lungs in the control I/R group (a) shows an alveolus (L) full of degenerated mitochondria (M), vesicles (V), protrusions of squamous epithelial cells (SEC) and tubular myelin(TM). The alveolus surrounded by capillaries (C) and a great alveolar cell (GAC) (× 12,000). (b) Shows two great alveoli cells (GAC). The alveolus (A) is narrowed, and the great alveolar cells appeared with disorganized mitochondria (M). (LB): Lamellar Bodies. Collagen fibers (CF) are accumulated in the septum (× 6000), in the EGCG group (c) shows a great alveolar cell (GAC) with lamellar bodies (LB) in the cytoplasm and normal appeared mitochondria (M). The alveoli are wide (A) and the squamous epithelial cells are thin without protrusions (SEC) (× 8000).

results in *de novo* production of pro-inflammatory cytokines and ROS [1]. Reperfusion of the ischemic intestine results in intestinal mucosal barrier impairment, leading to endotoxemia [9,32] that triggers a deleterious cascade including the production of ROS and further activates the intestinal macrophages and KCs. Activation of KCs, upregulates the production of inflammatory mediators such as interleukin-1β, interleukin-6, tumor necrosis factor-alpha and platelet activating factor [1,9]. Leukocytes are activated and accumulated in the liver, adhere to the sinusoidal endothelium through adhesion molecules, and trigger further production of ROS [8]. Intestinal I/R leads to liver injury mainly through activation of KCs and by PMNs aggregation and transmigration [8–9].

Intestinal I/R increases pulmonary vascular permeability and pulmonary edema, mainly through the aggregation and infiltration of leukocytes. As a result, intestinal I/R could lead to remote organ injury, ARDS and finally SIRS [8,13,14,33,34].

In this study, the impact of EGCG administration in attenuating the liver and lung injury induced by an experimental model of intestinal I/R, was investigated. Previous studies have shown that green tea extracts and its constituents including EGCG, could scavenge ROS and attenuate lipid peroxidation [3,19,35]. It has been shown that EGCG possesses

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an iron/copper-chelating activity, inhibiting generation of ROS [3,35].

Animals subjected to 60 min of intestinal ischemia followed by 120 min of reperfusion, showed higher ($p < 0.05$) MDA levels in liver compared to the sham operation group (Figure 1). Lung MDA levels were also elevated ($p < 0.05$) in the control I/R group compared to the sham operation group (Figure 1). The increased MDA levels in liver and lungs indicated significant lipid peroxidation, probably due to activated KCs and infiltrating PMN in liver and lungs, respectively. On the other hand, the animals pretreated with EGCG prior to intestinal I/R, showed a marked reduction of lipid peroxidation in liver and lungs, as this was documented by the significantly lower ($p < 0.05$) MDA levels compared to those of the control I/R group.

MPO levels in liver and lungs of the control I/R group were higher ($p < 0.05$) than those of the sham operation group (Figure 2). This lends support to the hypothesis that PMN infiltration is one of the main causes of lung and liver dysfunction following intestinal I/R, as indicated by MPO activity. In the group pretreated with EGCG, MPO levels in liver were markedly ($p < 0.05$) decreased reaching values similar to those of the sham operation group, probably indicating attenuation of PMN activation and accumulation. A significant ($p < 0.05$) decrease in lung MPO values was also observed in the EGCG treated I/R group, with values similar to the sham operation group, a finding suggesting attenuation of PMN activation and accumulation in the lungs.

Regarding AST and ALT levels in serum, there was a significant ($p < 0.05$) increase in the control I/R group compared to the sham operation group, indicating liver parenchyma injury elicited by intestinal I/R. AST and ALT values were significantly ($p < 0.05$) reduced in the I/R group pre-treated with EGCG, indicating protection of liver parenchyma (Figure 3).

In intestinal ischemia-reperfusion, hepatocytes may undergo various types of cell death such as necrosis, apoptosis and aponecrosis. In our animal model of intestinal ischemia-reperfusion, it appears that hepatocytes underwent aponecrosis as nucleus was condensed, cytoplasm was edematous, cell membrane was ruptured and remnants of organelles were free in the intracellular space (Figure 4a). Our observations are in accordance with Formigli et al. [36], who described the same pattern of cell death after hypoxic injury.

The results of this study show that intestinal I/R elicits lipid peroxidation and induces remote organ injury, especially in liver and lungs through activation of the KCs and migration of PMNs. The administration of EGCG prior to I/R, is likely to protect liver and lungs from lipid peroxidation elicited by intestinal I/R.

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References

[1] Towfigh S, Heisler T, Rigberg D, Hines J, Chu J, McFadden D, Chandler C. Intestinal ischemia and the gut-liver axis: An *in vitro* model. J Surg Res 2000;88:160–164.
[2] Ozden A, Tetik C, Bilgihan A, Calli N, Bostanci B, Yis O, Duzcan E. Antithrombin III prevents 60 min warm intestinal ischemia reperfusion injury in rats. Res Exp Med 1999;198:237–246.
[3] Wiseman SA, Balentine DA, Frei B. Antioxidants in Tea. Crit Rev Food Sci Nutr 1997;37:705–718.
[4] Haglund U, Bulkley GB, Granger DN. On the pathophysiology of intestinal ischemic injury. Acta Chir Scand 1987;153:321–324.
[5] Nakamura M, Ozaki M, Fuchinoue S, Teraoka S, Ota K. Ascorbic acid prevents ischemia/reperfusion injury in the rat small intestine. Transpl Int 1997;10:89–95.
[6] Tetik C, Ozden A, Calli N, Bilgihan A, Bostanci B, Yis O, Bayramoglu H. Cytoprotective effect of trimetazidine on 60 minutes of intestinal ischemia/reperfusion injury in rats. Transpl Int 1999;12:108–112.
[7] Yamamoto S, Tanabe M, Wakabayashi G, Schimazu M, Matsumoto K, Kitajima M. The role of tumor necrosis factor-a and interleukin-1β in ischemia/reperfusion injury of the rat small intestine. J Surg Res 2001;99:134–141.
[8] Horie Y, Ishii H. Liver dysfunction elicited by gut ischemia/reperfusion. Pathophysiology 2001;8:11–20.
[9] Horie Y, Wolf R, Russell J, Shanley TP, Granger N. Role of Kupffer cells in gut ischemia/reperfusion—induced hepatic microvascular dysfunction in mice. Hepatology 1997;26:1499–1505.
[10] Clavien PA, Camargo Jr., CA, Gorczynski R, Washington MK, Levy GA, Greig PD. Acute reactant cytokines and neutrophil adhesion after warm ischemia in cirrhotic and non-cirrhotic human liver. Hepatology 1996;23:1456–1463.
[11] Suzuki S, Toledo-Pereyra LH, Rodrigues F, Lopez F. Role of Kupffer cells in neutrophil activation and infiltration following total hepatic ischemia and reperfusion. Circ Shock 1994;42:204–209.
[12] Decker K. Biologically active products of stimulated liver macrophages (Kupffer cells). Eur J Biochem 1990; 192:245–261.
[13] Tullis MJ, Brown S, Gewertz BL. Hepatic influence on pulmonary neutrophil sequestration following intestinal ischemia/reperfusion. J Surg Res 1996;66:143–146.
[14] Cavriani G, Oliveira-Filho RM, Trezena AG, da Silva ZL, Domingos HV, de Arruda MJC, Jancar S, de Lima WT. Lung microvascular permeability and neutrophil recruitment are differently regulated by nitric oxide in a rat model of intestinal ischemia/reperfusion. Eur J Pharmacol 2004; 494:241–249.
[15] Boros M, Takaichi S, Hatanaka K. Ischemic time-dependent microvascular changes and reperfusion injury in the rat small intestine. J Surg Res 1994;59:311–320.
[16] Ward DT, Lawson SA, Gallagher CM, Conner WC, Shea-Donohue T. Sustained nitric oxide production via L-arginine administration ameliorates effects of intestinal ischemia/reperfusion. J Surg Res 2000;89:13–19.
[17] Lee S, Suh II, S, Kim SP. Protective effects of the green tea polyphenol (−)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. Neurosci Lett 2000;30:191–194.
[18] Kohri T, Nanjo F, Suzuki M, Seto R, Matsumoto N, Yamakawa M, Hojo H, Hara Y, Desai D, Amin S, Conaway CC, Chung FL. Synthesis of (−)-[4-3H] Epigallocatechin gallate and its metabolic fate in rats after intravenous administration. J Agric Food Chem 2001;49:1042–1048.
[19] Higuchi A, Yonemitsu K, Koreeda A, Tsunenari S. Inhibitory activity of epigallocatechin gallate (EGCG) in paraquat-induced

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microsomal lipid peroxidation—a mechanism of protective effects of EGCG against paraquat toxicity. Toxicology 2002; 183:143–149.

[20] Wang H, Provan GJ, Helliwell K. Tea flavonoids: Their functions, utilization and analysis. Trends Food Sci Tecnhol 2000;11:152–160.

[21] Ahmed S, Rahman A, Hasnain A, Lalonde M, Goldberg VM, Haqqi TM. Green tea polyphenol Epigallocatechin-3-gallate inhibits the IL-1β-induced activity and expression of cyclooxygenase-2 and nitric oxide synthase-2 in human chondrocytes. Free Radic Biol Med 2002;33:1097–1105.

[22] Jimenez-Lopez JM, Cederbaum AI. Green tea polyphenol epigallocatechin-3-gallate protects HepG2 cells against CYP2E1-dependent toxicity. Free Radic Biol Med 2003;36: 359–370.

[23] Chen L, Lee MJ, Yang CS. Absorption, distribution, and elimination of tea polyphenols in rats. Drug Metab Dispos 1997;25:1045–1050.

[24] Chan MMY, Fong D, Ho CT, Huang HI. Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. Biochem Pharmacol 1997;154: 1281–1286.

[25] Paquay JB, Haenen B, Stander GR, Wiseman SA, Tijburg LB, Bast A. Protection against nitric oxide toxicity by tea. J Agric Food Chem 2000;48:5768–5772.

[26] Committee on Care and Use of Laboratory Animals. Guide for the care and use of laboratory animals. Institute of Laboratory Animal Resources, National Research Council, Washington: D.C.; 1985.

[27] Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ, Trakatelis AG. Rapid, sensitive and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food and feedstuff samples. J Agric Food Chem 1994;42:1931–1936.

[28] Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 1984;87:1344–1350.

[29] Grisham MB, Hernandez LA, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. Am J Physiol 1986;251:G567–G574.

[30] Matsumura F, Yamaguchi Y, Goto M, Ichiguchi O, Akizuki E, Matsuda T, Okabe K, Liang J, Ohshiro H, Iwamoto T, Yamada S, Mori K, Ogawa M. Xanthine oxidase inhibition attenuates Kupffer cell production of neutrophils chemoattractant following ischemia—reperfusion in rat liver. Hepatology 1998;28:1578–1587.

[31] Steel RGD, Torrie JH. Principles and procedures of Statistics. A Biometrical Approach. 2nd ed. New York: McGraw-Hill Book Co. 1980.

[32] Sun Z, Wang X, Lasson A, Borjesson A, Leveau P, Haraldsen P, Andersson R. Roles of platelet-activating factor, interleukin-1β and interleukin-6 in intestinal barrier dysfunction induced by mesenteric arterial ischemia and reperfusion. J Surg Res 1999;87:90–100.

[33] Savas MC, Ozguner M, Ozguner IF, Delibas N. Splenectomy attenuates intestinal ischemia/reperfusion-induced acute lung injury. J Pediatr Surg 2003;38:1465–1470.

[34] Turnage RH, Wright JK, Iglesias J. Intestinal reperfusion-induced pulmonary edema is related to increased pulmonary inducible nitric oxide synthase activity. Surgery 1998;124:457–463.

[35] Zhong Z, Froh M, Connor HD, Li X, Conzelmann LO, Mason RP, Lemasters JJ, Thurman RG. Prevention of hepatic ischemia/reperfusion injury by green tea extract. Am J Physiol 2002;283:G957–G964.

[36] Formigli L, Papucci L, Tani A, Schiavone N, Tempestini A, Orlandini GE, Capaccioli S, Orlandini SZ. Aponecrosis: Morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. J Cell Physiol 2000;182:41–49.

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