Effects of U-74389G (21-Lazaroid) and Ascorbic Acid on Liver Recovery After Acute Ischemia and Reperfusion in Rats

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Abstract. Background/Aim: The free radical-scavenging effects of the lazaroid U-74389G have been shown in several experimental models to protect the liver from ischemia/reperfusion (I/R), however, the mechanism of cytoprotection is not fully understood. Similar findings were observed when ascorbic acid was administered. This study investigates the effects of infusion of lazaroid U-74389G and ascorbic acid on cytokines and liver structure in a liver I/R rat model. Materials and Methods: Sixty male Wister rats, weighting 220-290 g, were used in the study. Six experimental groups were formed: Group 1 (control group): ischemia for 30 min and reperfusion for 60 min; group 2 (control group): ischemia for 30 min and reperfusion for 120 min; group 3: ischemia for 30 min, intraportal injection of ascorbic acid, and reperfusion for 60 min; group 4: ischemia for 30 min, ascorbic acid administration, and reperfusion for 120 min; group 5: ischemia for 30 min, U-74389G administration, and reperfusion for 60 min; and group 6: ischemia for 30 min, U-74389G administration, and reperfusion for 120 min. Tissue and blood sampling took place upon completion of each model’s reperfusion. U-74389G was administered at 10 mg/kg animal body weight and ascorbic acid at 100 mg/kg. Anesthesia was induced with ketamine and xylazine. Surgery was performed through a midline laparotomy. The portal vein and the common hepatic artery were isolated and prepared for occlusion. Blood samples and wedge liver biopsies were taken to measure levels of liver enzymes, cytokines and for microscopic analysis upon completion of reperfusion once for each model. Results: Histopathological evaluation revealed a statistically significant reduction in the degree of necrosis of liver tissue in the treated groups compared to the control groups 1 and 2 [groups 3, 5 (p=0.010) and 4, 6 (p<0.0005)]. On the other hand, tissue malondialdehyde levels (MDA) were statistically significantly increased only between control group 2 and groups 4, 6 (p<0.0005). There was no statistically significant difference in tumor necrosis factor-α between groups. As for liver enzymes, only alkaline phosphatase (ALP) and gamma-glutamyl transferase (gGT) were statistically significantly reduced in treated groups 3 and 5 (ALP: p=0.027, and gGT: p=0.002) and 4 and 6 (ALP: p=0.004, and gGT: p=0.015) compared to control groups 1 and 2. Conclusion: Based on histological data and the reduction of some of the liver enzymes, in spite of a rise of malondialdehyde, in this rat model, administration of U-74389G in liver ischemia/reperfusion (I/R) injury has potential in attenuating liver damage.

Ischemia/reperfusion (I/R) injury remains a significant factor in the morbidity and mortality of liver resection, trauma, and transplantation (1). The development of therapeutic strategies that reduce I/R liver injury will have a major impact on patient outcome (2, 3). Hepatic I/R injury consists of a sequence of cellular and humoral events that finally lead to parenchymal and non-parenchymal cell death (Figure 1). It is of utmost importance to the outcome of liver transplantation and liver resections. There is ample evidence that the key role in such inflicted injury is ascribed to reactive oxygen species (ROS) generated mainly by Kupffer cells and neutrophils during reperfusion, with the participation of endothelial cells and hepatocytes. ROS can cause significant damage to cellular membranes...
and genomic material. The process culminates with the initiation of several inflammatory mediators.

On the other hand, cells possess very potent, enzymatic and non-enzymatic anti-oxidant systems capable of mitigating oxidant stress or scavenging ROS, thus preserving to some extent the cellular redox state. When the imbalance between oxidant stress and anti-oxidant systems predominates, cell death ensues. The spectrum of therapeutic options for liver I/R injury includes a plethora of anti-oxidant agents, natural and synthetic, and extends to genetic modifications (4).

The 21-aminosteroids, or lazaroids, are a recently developed (after 1980) novel series of lipid peroxidation inhibitors, whose therapeutic potential has been extensively studied. Although the components of this family (Table I) are derived from glucocorticoids, they lack glucocorticoid and mineralcorticoid activities. Lazaroids are able to scavenge lipid peroxyl and phenoxyl radicals, to inhibit iron-dependent lipid peroxidation and to protect against peroxynitrite-induced cell toxicity (5, 6).

The basis for the development of 21-a minosteroids was that they specifically localize in (or very near) hydrophobic or lipid domains of biological membranes (the presumed sites at which these drugs inhibit lipid peroxidation) and induce a strong increase in lipid packing order (7). The evidence that the lazaroid U-74006F blocks release of arachidonate (which produces vasogenic edema) from injured cell membranes further supports this hypothesis (5).

Since peroxidative damage to membranes may be prevented by lipophilic drugs that have membrane-stabilizing effects, the anti-lipid peroxidation effect of lazaroids might be exerted through cooperative mechanisms, such as a radical-scavenging and an alteration of cell membranes.

The strong free radical-scavenging effect of the lazaroid U-74389G has been shown in several experimental models. For example, U-74389G had beneficial effects against endotoxin-induced shock in rats (8) and mice (9), protected against liver, lung and heart ischemia-reperfusion injury (10-12), and prevented paraquat-induced damage in the rat brain (13) (Table II).

Ascorbic acid [(5R)-{(1S)-1,2-dihydroxyethyl}-3,4-dihydroxyfuran-2 (5H)-one] is a naturally occurring organic compound with antioxidant properties. It is a white solid, but impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. Ascorbic acid is found in plants and animals where it is produced from glucose. Its biosynthesis starts with the formation UDP-glucuronic acid.

As a mild reducing agent, ascorbic acid degrades upon exposure to air, converting oxygen to water. The redox reaction is accelerated by the presence of metal ions and light. It can be oxidized by one electron to a radical state or doubly-oxidized to the stable form called dehydroascorbic acid. Ascorbate can terminate chain radical reactions by electron transfer owing to the stability of its own radical ion. However, being a good electron donor, excess ascorbate in the presence of free metal ions cannot only promote but also initiate free radical reactions, thus making potentially dangerous pro-oxidative compounds in certain metabolic contexts.

The efficacy of ascorbic acid has been shown in several experimental models. For example, ascorbic acid attenuated myocardial injury and protected cardiac function in rats (14) and had significant protective effects on rat reperfusion liver injury by attenuating hydroxyl radical and NO release (15).

The use of ascorbic acid also reduced the level of

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**Figure 1. Pathophysiology of liver/ischemia reperfusion injury.** During the early sub-acute phase of reperfusion which covers the first 2-3 h, the activation of Kupffer cells is the main feature. The factors responsible for this activation are hypoxia in combination with complement and CD4+ T-lymphocytes. During the late phase (>6 h) of reperfusion, the inflammatory mediators augment cellular and vascular dysfunction by releasing neutrophil chemoattractants and inducing expression of adhesion molecules. Neutrophil accumulation augments oxidative stress via Reactive Oxygen Species (ROS) and protease release (4).

**Table I. Lazaroids most frequently used in experimental protocols.**

<table>
<thead>
<tr>
<th>Lazaroid</th>
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<tbody>
<tr>
<td>U-83836E</td>
</tr>
<tr>
<td>U-74500A</td>
</tr>
<tr>
<td>U-75412E</td>
</tr>
<tr>
<td>U-74006F</td>
</tr>
<tr>
<td>U-74389F</td>
</tr>
<tr>
<td>U-74389G</td>
</tr>
<tr>
<td>U-83836E</td>
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</table>
mitochondrial damage during I/R as a result of decreased post-ischemic oxidant stress (16) (Table III).

The aim of the present study was to investigate the effects of infusion of lazaroid U-74389G and ascorbic acid on cytokines and liver structure in a liver I/R rat model.

**Materials and Methods**

All experimental procedures in this study were assessed and approved by an Institutional Ethical Review Board and all animals received humane care in accordance with institutional guidelines. The experiments were approved by Experimental Research Center ELPEN and the veterinary authorities of East Attica Region, in accordance with Greek Law No. 160 (A-64, May 1991), European Union regulations, and the principles of the Helsinki Declaration [in accordance with institutional guidelines for the care and use of laboratory animals consistent with the guidelines of the European Community Council Directive on the use of laboratory animals in experiments (86/609/EEC)]. (License Reference Number: 1088, Veterinary Authority of East Attica Region, Ioannis Kappas.)

The 21-aminosteroid U-74389G (CAS number 153190-29-5; pregn-a,1,4,9(11)-triene-3,20-dione,21-[4-(4,6-di-pyrrolidinyl-1-piperazinyl)-2-butenedioate; SIGMA - ALDRICH® Company, St Louis, Missouri, USA) was used at a dose of U-74389G 10 mg/kg animal body weight i.v. This dose of the lazaroid was determined based on previous experience in the same laboratory with the use of U-74389G (20); the same dose has also been used in various other studies (21, 42) and seems to be beneficial and the most important studies about the mechanism of action in liver of 21-aminosteroids were carried out at this dose (14, 15). Ascorbic acid was used at a dose of 200 mg/kg i.v. (31).

Table II. *Mechanisms of action/results of lazaroids in experimental models in rats using lazaroids.*

<table>
<thead>
<tr>
<th>Mechanism of action/effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated structural damage.</td>
<td>Carrea et al. (1992) (33)</td>
</tr>
<tr>
<td>Reduced expression of cytokines, inducible nitric oxide synthase, and Major Histocompatibility Complex (MHC) antigens.</td>
<td>Fantini et al. (1996) (34)</td>
</tr>
<tr>
<td>Enhanced both systolic and diastolic left ventricular recovery.</td>
<td>Weir et al. (1992) (35)</td>
</tr>
<tr>
<td>Protected lipid component of cell membranes from peroxidation by reactive oxygen metabolites.</td>
<td>Van Ye et al. (1992) (36)</td>
</tr>
<tr>
<td>Affected structural and functional aspects of cellular membrane injury on reperfusion</td>
<td>Stojadinovic et al. (1999) (37)</td>
</tr>
<tr>
<td>Acted as a free radical phagocyte and antagonized iron dependent lipid oxidation.</td>
<td>Squadrì et al. (1996) (38)</td>
</tr>
<tr>
<td>Effective inhibition of lipid peroxidation.</td>
<td>Javier de Oca et al. (1998) (39)</td>
</tr>
<tr>
<td>Significantly reduced neutrophil infiltration, prevented increase of leukotriene B4 and prostaglandin E2.</td>
<td>Fotiadis et al. (2003) (20)</td>
</tr>
<tr>
<td>Lipid peroxidation inhibitor; it has antishock and endothelial protective actions.</td>
<td></td>
</tr>
<tr>
<td>Lipid peroxidation inhibitor; protects intestinal graft, reduces bacterial translocation.</td>
<td></td>
</tr>
<tr>
<td>Statistically significant reduction in the concentration of small intestine tissue malon dialdehyde.</td>
<td></td>
</tr>
<tr>
<td>Source; (6)</td>
<td></td>
</tr>
</tbody>
</table>

Table III. *Mechanisms of action of ascorbic acid in experimental models in rats using ascorbic acid.*

<table>
<thead>
<tr>
<th>Mechanisms of action/Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved cardiac function and significantly reduced cardiac injury (reduction in Troponin I and plasma creatine kinase – MB) by scavenging hydroxyl radical and reducing lipid peroxidation.</td>
<td>Hsu C et al. (2012) (14)</td>
</tr>
<tr>
<td>Attenuation hydroxyl radical and NO release and therefore protective effects.</td>
<td>Wang NT et al. (2009) (15)</td>
</tr>
<tr>
<td>Reduction of the level of mitochondrial damage during I/R .</td>
<td>Lee et al. (2007) (16)</td>
</tr>
<tr>
<td>Attenuation of serum aminotransferase levels and lipid peroxidation.</td>
<td>Seo et al. (2002) (17)</td>
</tr>
<tr>
<td>Increased the BH4/dihydrobiopterin (BH2) ratio and inhibit uncoupling of NOS.</td>
<td>Okazaki T et al. (2011) (19)</td>
</tr>
</tbody>
</table>

Table IV. *Histopatohological evaluation of necrosis between groups.*

<table>
<thead>
<tr>
<th>Pathology score</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control)</td>
<td>10</td>
<td>3.10</td>
<td>1.66</td>
<td>0.01</td>
</tr>
<tr>
<td>Group 3 (ascorbic acid)</td>
<td>10</td>
<td>2.00</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Group 5 (U-74389G)</td>
<td>10</td>
<td>1.30</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Group 2 (control)</td>
<td>10</td>
<td>3.90</td>
<td>1.10</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Group 4 (ascorbic acid)</td>
<td>10</td>
<td>1.78</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>Group 6 (U-74389G)</td>
<td>10</td>
<td>1.13</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

CK – MB; creatine kinase MB Isoenzyme; NO; mono-nitrogen oxide; I/R; ischemia – reperfusion; MDA; malondialdehyde; CREA; creatinine; BUN; blood urea nitrogen; LDH; lactate dehydrogenase; NOS; nitric oxide synthase; BH4; tetrahydrobiopterin; BH2; dihydrobiopterin.
acids was purchased from HOFFMANN LA ROCHE® Company (90% content; ARG CHEM s.a. Basel, Switzerland).

**Animal model of liver I/R injury.** Sixty male Wistar rats weighing 220-290 g (initial body weight) were used after a period of acclimatization. The animals were purchased from EKEFE DIMOCRITOS (Ag. Parakevi, Athens, Greece). Animals had free access to laboratory chow and tap water. They were maintained on a 12/12 h light-dark cycle and housed in an animal room, where temperature (22-24°C) and humidity (65-75%) were controlled.

**Experimental groups:** Male Wistar rats were randomly distributed into six groups (10 animals each) [n=60], such that there were no statistically significant differences among the mean body weight of groups. Group 1: (control group): ischemia for 30 min and reperfusion for 60 min; group 2: (control group) ischemia for 30 min and reperfusion for 120 min; group 3: treated group, subjected to 30 min ischemia, ascorbic acid 100 mg/kg i.v., and reperfusion for 60 min; group 4: treated group, subjected to 30 min ischemia, ascorbic acid 100 mg/kg i.v., and reperfusion for 120 min; group 5: treated group, subjected to 30 min ischemia, U74389G 10 mg/kg i.v., and reperfusion for 60 min; group 6: treated group, subjected to 30 min ischemia, U74389G 10 mg/kg i.v., and reperfusion for 120 min. Tissue and blood sampling were carried out at the completion of reperfusion in each group. The effect of I/R injury and its modulation by ascorbic acid or the 21-aminosteroid U74389G was assessed reviewing liver function, adhesion molecules expression, inflammatory mediators and liver histology.

**I/R Protocol:** After 8 h fasting, the animals were anaesthetized with a ketamine/xylazine mixture at 100/10 mg/kg body weight intramuscularly. Supplementary doses were given, if necessary, throughout the exposed peritoneum. At the end of the surgical procedures, carprofen was administered at 2-5 mg/kg i.m. and acemycin at 10 mg/kg i.m. Following ischemia, ascorbic acid or the 21-aminosteroid U74389G was infused into the portal vein while controls received equal volumes of Ringer’s lactate solution.

We performed a midline laparotomy, with dissection of the liver area (Figure 2), selective clamping of vascular structures (portal vein, hepatic artery) (Figure 3), and biliary duct (medial and left lateral lobes, about 60% of hepatic parenchyma) with a vascular microclamp, thus inducing ischemia for 30 min (Figure 4). Throughout the procedure, the surgical area was perfused with saline serum to avoid dehydration. After 30 min, the clamp was removed, which allowed tissue reperfusion for 60 or 120 min according to which group the model belonged to. Drugs were administered through direct puncture of the inferior vena cava; U74389G was injected at 10 mg/kg and ascorbic acid at 100 mg/kg.

Control operated animals were only subjected to anesthesia and laparotomy. Blood samples (4-5 ml) were obtained through direct puncture of the aorta. At the end of the procedure, animals were sacrificed.

**Sampling.** Blood samples and wedge liver biopsies were taken to measure levels of liver enzymes, cytokines and histology, and for microscopic analysis at the completion of reperfusion once for each model. Collected blood was centrifuged, and serum stored at −80°C for analysis.

**Serum immunoassay for transaminases and cytokines.** Serum was obtained by blood centrifugation at a relative Centrifugal Force (RCF) or G-force of 958 g (3,000 rpm) for 10 min and stored at −80°C. Levels of liver serum enzymes alanine aminotransferase (SGOT), aspartate aminotransferase (SGPT), alkaline phosphatase (ALP), creatinine, and total and direct bilirubin (tBil, dBil) were determined using an automatic analyzer. All blood samples were analyzed blindly having been randomly assigned code numbers. All determinations were performed in duplicate with less than 10% variability among duplicate wells and means were estimated. TNFα was measured with the Quantikine ELISA kit (Porcine TNFα, R & D Systems, Tools for Cell Biology Research™, For Research Use Only, Catalog Number PTA00).

**Measurements of lipid peroxidation in liver.** A piece of liver (approximately 0.5 g) was processed to obtain a homogenate and to measure malon dialdehyde (MDA) levels, as described by Boada et al. (1999) (modified from Esterbauer and Cheeseman 1990), on a Beckman DU7400 spectrophotometer (Beckman Instruments Inc., CA, USA) (40).

Tissue samples were rinsed with ice-cold isotonic saline before homogenization, which was carried out using 20 mmol/l Tris-buffered (pH 7.4) and an Ultra-Turrax (Ika-Labortechnik, Staufen - Germany) blender. One milliliter of buffer was used for 0.1 g of tissue. Ten milliliters of 500 mmol/l butylated hydroxytoluene was added to 1 ml of tissue homogenate to prevent sample oxidation. The homogenate was centrifuged at a relative Centrifugal Force (RCF) or G-force of 958 g (3,000 rpm) at 4°C for 10 min. Then 0.2 ml of tissue homogenate supernatant and 0.65 ml of diluted MPI reagent (technetium tc-99m succimer kit) were added to a polypropylene microcentrifuge tube (supplied by GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). The mixture was vortexed and then 0.15 ml of 12 mol/l HCl was added. Tubes were incubated at 45°C for 60 min and centrifuged at a relative Centrifugal Force (RCF) or G-force of 3,830 g (3,000 rpm) for 15 min. Then the optical density of 0.8 ml of the supernatant was measured at 586 nm. MDA standards for the standard curve were made by dilutions of the stock 10 mm tetramethoxypropane solution.

**Histological analysis for I/R injury.** Tissue specimens were fixed in formalin (4%) and embedded in paraffin according to standard histological procedures. Four-micron sections of paraffin-embedded tissue samples from each case were sectioned, deparaffinized, dehydrated, and finally subjected to conventional hematoxylin and eosin staining (H&E). Histomorphological alterations were assessed using a four-mark scoring system: 0: normal, 1: minimal alterations of less than 25% (of the field), 2: moderate alterations of 25-50% (of the field), 3: severe alterations of over 50% (of the field), and separate scores were obtained for each of the following: vascular congestion (vascular dilatation demonstrating erythrocytes within centrilobular veins), steatosis (vascular accumulation of free fatty acids within hepatocytes) and hepatocellular necrosis (hepatocytes with microscopic aspects of necrosis such as nuclear pyknosis, karyorrhexis and karyolysis without pericellular inflammatory infiltrate). Specimens were examined under a Nikon eclipse 50i microscope. (Nikon Instruments Europe BV, Amsterdam, The Netherlands). Five randomly selected fields in each specimen were examined. Histomorphological analysis was performed by one pathologist who was blinded to the clinical and pathological information (22, 23).

**Statistical analysis.** Data are expressed as the mean±standard deviation (S.D.) or median (in case of violation of normality) for continuous variables and as percentages for categorical data. The
Kolmogorov–Smirnov test was utilized for normality analysis of the parameters. The comparison of variables between the three groups with intervention (control vs. U-74389G vs. ascorbic acid) was performed using the one-way ANOVA model. Pairwise comparisons performed using the Bonferroni test. Kruskal–Wallis test and Mann–Whitney test were used in cases of violation of normality. The comparison of the variables between the two times of reperfusion (60 vs. 120 min) for each intervention group was performed using the independent samples t-test) and Mann–Whitney test in cases of violation of normality. All tests are two-sided, statistical significance was set at $p<0.05$ but borderline differentiation is also mentioned ($0.05<p<0.1$). All analyses were carried out using the statistical package SPSS version 16.00 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA).

**Results**

*Histology.* The histopathological evaluation revealed a statistically significant difference ($p=0.01$) in necrosis between control group 1 and groups 3 (ascorbic acid) and 5 (U-74389G). There was also a statistically significant difference ($p<0.0005$) in necrosis between control group 2 and groups 4 (treated with ascorbic acid) and 6 (U-74389G). (Figure 8) (Table IV). All the other histological parameters studied did not statistically significantly differ between control and therapy groups. There was an obvious reduction in the number of hepatocytes with microscopic aspects of necrosis such as nuclear pyknosis, karyorrhexis and karyolysis without pericellular inflammatory infiltrate in treated groups (Figures 7-11).

*MDA levels (Figure 6 and Table V).* Levels of MDA were measured for evaluation of lipid peroxidation in rat liver. Variables MDA-ΔA and MDA-μM were analyzed. MDA has been identified as the product of lipid peroxidation. During the process of measuring its levels the absorbance of the sample was determined at 586 nm against a blank that contained all the reagents except the serum. This provided us the net Abs (Abs-Abs Blank). Based on the Bradford reaction we calculated the total protein of each aliquot. MDA-ΔA therefore is the difference in the MDA levels between the sample and the blank specimen that is further adjusted based on the total protein per each aliquot and the MDA reference curve in order to provide us the exact value of the MDA in μmol/mL (MDA-μM). The analysis did not show statistically significant differences between control group 1 and therapy groups 3 and 5. However, there was a statistically significant increase ($p<0.0005$) in MDA-ΔA and MDA-μM between control group 2 and groups 4 (treated with ascorbic acid) and 6 (treated with U-74389G) (Table V).
Figures 5 and 6. Median values of Serum glutamic oxaloacetic transaminase (SGOT) (mg/dl), serum glutamic-pyruvic transaminase (SGPT) (mg/dl), Alkaline phosphatase (ALP) (IU/L) and tumor necrosis factor alpha, (TNFα) (pg/ml) (A) and Creatinine (Cre) (mg/dl), Total Bilirubin (tBil) (mg/dl), Direct bilirubin (dBil) (mg/dl), gamma-glutamyl transferase (γGT) (IU/L), Malondialdehyde difference (MDA-ΔA) and Malondialdehyde levels (MDA-μM) (B) in different groups. MDA: ΔA net MDA has been identified as the product of lipid peroxidation. During the process of measuring its levels the absorbance of the sample was determined at 586 nm against a blank that contained all the reagents except the serum. This provided us the net Abs (Abs - Abs Blank). Based on Bradford reaction we calculated the total protein of each aliquot. MDA-ΔA therefore is the difference in the MDA levels between the sample and the blank specimen that is adjusted further based on the total protein per each aliquot and the MDA reference curve in order to provide us the exact value of the MDA in μmol/mL (MDA-μM).
TNFα (Figure 5). The comparison of TNFα values (in pg/ml) showed absence of any statistical significant differences between groups (Table VI).

Serum transaminases and other liver enzymes (Figures 5 and 6). There was an absence of or only borderline statistically significant differences in SGOT, SGPT, tBil and dBil between control groups and groups 3 and 4 (treated with ascorbic acid) and 5 and 6 (treated with U-74389G) although their levels were mainly reduced.

The analysis revealed statistically significant reductions in ALP, gGT and creatinine: ALP, $p=0.027$ for groups 3 and 5 and 0.002 for groups 4 and 6 compared to their respective controls; gGT, $p=0.004$ and 0.015, respectively creatinine,
Discussion

I/R liver injury is a crucial clinical problem following liver surgery or liver transplantation. Although the consequences of hepatic I/R injury have been widely studied, all the effects connected with primary malfunctioning and non-functioning grafts are not yet well-known (10). Previous data show that after ischemia there is an increase in serum hepatic transaminases, this increase being larger when there is tissue reperfusion, and it is even greater as reperfusion time increases (42).

Previous studies have investigated the effect of lazaroids on reducing damage from ischemia-reperfusion injury in different organs and in different experimental models (20, 21-28). Cosenza et al. studied the effect of lazaroid U-74000F in ex vivo isolated pig livers and found that it enhanced the resistance of the liver to damage after I/R (24). Later, Iwanami et al. studied the effect of various lazaroids on liver ischemia in dogs with improved survival rates to the treated group (41). We reviewed both studies in designing our rat experiments using U-74389G in liver I/R injury. It is also important to point-out that molecules from the same family of organic synthesis have different properties, as well as different final beneficial effects. Our study describes the beneficial effect of U-74389G in acute I/R in a rat model.

The protective effect of lazaroids is exerted mainly through their free radical-scavenging qualities and de-granulation of neutrophils. The administration of anti-oxidants at different time points has also been studied; it was found that substances with possible anti-oxidant effects could be administered before ischemia or at the end of ischemia before reperfusion (20, 21, 24-28, 29). However, in clinical practice, it is very rare to begin treatment before the onset of ischemia. Nevertheless, in other studies the lazaroid was administered before ischemia (12, 13). In our study, lazaroid administration was at the end of ischemia; therefore, one cannot make any conclusions regarding the effect of the time of administration, particularly when both treatments had positive effects on hepatic injury. We also believe that administration of lazaroids at the end of ischemia may be more similar to clinical practice if any in the future, particularly in emergency hepatic surgery. However, this is a point needing more attention.

Activated polymorphonuclear leukocytes, which accumulate after reperfusion in the capillaries and venules of the tissue, are a potential source of free oxygen radicals. Stimulation of neutrophils induces the release of ROS, mediating tissue damage (Figure 1). Histological evaluation is performed to check the effect of post-reperfusion tissue damage, suggested by changes such as the activation of Kupffer cells, portal infiltration, intralobular necrosis, intracelular swelling, reaction of the liver capsule, subcapsular necrosis, hemorrhage, and hyperemia of sinusoidal spaces (6). In this study, liver injury was evaluated by histopathological examination that had portal infiltration as an index of inflammation of the liver tissue.

The administration of U-74389G led to a lower degree of portal infiltration in the therapy groups (see pathology examination section). This finding shows the positive effect of U-74389G administration on the hepatic parenchyma. However, it should be mentioned that this does not necessarily have direct implications on the protection of the hepatic parenchyma (i.e. of hepatocytes). Parameters reflecting the severity of hepatocellular injury were used to assess the damage to the liver parenchyma. In previous studies, it was shown that transaminases were significantly lower in the group receiving lazaroids U-74000F and U-74500A during the early
postoperative period. In the present study, the plasma levels of SGOT and SGPT were mainly lower in treated groups (i.e. groups 3-6), but there was no statistically significant reduction in the therapy groups compared to the control groups. On the other hand, there was a statistically significant reduction in treated groups in the levels of ALP and gGT.

Lazaroids can also have a protective effect on endotoxic shock by either reducing the oxidant damage or attenuating TNFα production (31). The effect of U-74389G on TNFα production was evaluated and our data suggest that although TNFα values were higher for treated groups, there was an absence of any statistical significance. The effect of lazaroid on hepatic tissue, as indicated through MDA formation in liver, is shown mainly through the reduction of MDA values. However, in our experiment there was a statistically significant rise of MDA-AA and MDA-μM values after reperfusion when administering U-74389G or ascorbic acid.

Our study, however, had the limitations in that there was only a 1 to 2-h period of liver reperfusion. Further investigations consequently are needed to assess the long-term effectiveness of lazaroid U-74389G on reperfused liver function, using large animal models, before a clinical trial could be justified.

To our knowledge, this is the first study of a specific animal setting that tests the effect of lazaroid U-74389G on liver vascular occlusion using a rat model in vivo, evaluating impact on histology and presenting measurements of early hepatocellular injury. The results of the histological study, together with the reduction of some serum transaminases and other liver enzymes, despite the MDA rise, indicate that U-74389G and ascorbic acid have potential in attenuating liver damage from I/R injury.

Acknowledgements

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Conflicts of Interest

All the Authors confirm that there were no financial or personal relationships with other people or organizations that could inappropriately influence (bias) the work.

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