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Does calcium dobesilate protect against intestinal ischemia-reperfusion injury induced in rats?

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Abstract. – OBJECTIVE: In this study, we investigated whether the administration of calcium dobesilate (CD) affects oxidative stress markers and histopathological outcomes in a rat model of intestinal ischemia-reperfusion (IR) injury.

MATERIALS AND METHODS: This study was conducted with 30 male Wistar rats. The rats were randomly assigned to three groups as follows: a sham group (n = 10), an IR group (n = 10), and an IR + CD group (n = 10). In the sham group, superior mesenteric artery (SMA) dissection alone was performed during laparotomy. In the IR group, the procedure included SMA occlusion for 60 min, followed by reperfusion for 60 min. In the IR + CD group, CD (100 mg/kg/day) was additionally given for two days before laparotomy by intragastric lavage. In all the rats, 2 ml of blood were drawn, and an ileal segment (approximately 2 cm in size) was removed to evaluate oxidative stress markers. The ileal segment removed was divided into two pieces, and one piece was reserved for histopathological evaluation.

RESULTS: Compared to the other groups, both serum and tissue oxidative stress indices were lower in the IR + CD group. The decrease was due to CD increasing the total antioxidant capacity. Moreover, the histological analysis showed that CD reduced tissue injury.

CONCLUSIONS: CD may exert a protective effect against intestinal IR injury by increasing antioxidant capacity.

Key Words:
Calcium dobesilate, Intestinal ischemia-reperfusion injury, Histopathological injury.

Introduction

Acute mesenteric ischemia (AMI) is a devastating clinical condition characterized by reduced blood flow caused by an arterial thrombus in the superior mesenteric artery (SMA), inferior mesenteric artery or truncus coeliacus[1]. AMI comprises approximately 2% of gastrointestinal diseases requiring immediate surgical intervention[1,2]. The mortality remains at 60-80%, despite advances in diagnosis and management[3].

Intestinal ischemia-reperfusion injury (IRI) is defined as cellular injury secondary to ischemia and reperfusion of the intestine, and it plays a major role in mortality of AMI[1]. Ischemia is defined as a marked reduction in the blood flow required by tissues and organs, and reperfusion is defined as restoration of blood flow to tissues[4]. But during this period, proinflammatory cytokine release and reactive oxygen species (ROS) production, which is cause to tissue injury are increased[5,6].

Oxidative stability is defined as the equilibrium between ROS production and elimination[7]. Oxidative stress is defined as an imbalance between ROS and antioxidant production involved in ROS inactivation[8]. The elevated level of oxidative stress causes injury to cellular lipids, proteins and deoxyribonucleic acid (DNA), leading to the development of numerous diseases[8,9]. ROS may lead to an inflammatory response, including complement activation, cytokine release and leukocyte activation[7].

Calcium dobesilate (CD) is a pharmacological agent used in the treatment of many diseases because of its vasoprotective and antioxidant features[10]. The antioxidant properties of CD are attributed to reduced lipid peroxidation caused by ROS and decreased release of inflammatory cytokines, such as platelet activating factor (PAF)[11].

In the present study, we aimed to investigate whether the antioxidant properties of CD influence the release of serum and tissue oxidative

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markers and confer protection against tissue damage in IRI induced in rats.

Materials and Methods

Animals

The study protocol was approved by the Local Ethics Committee on Animal Studies of Dicle University (protocol no: 12.02.2013/04-9). The care of the rats and all surgical procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals".

Thirty male Wistar rats (weighing 180-240 g) were supplied by the Animal Experiment Laboratory of Dicle University (Diyarbakir, Turkey). The rats were housed in stainless steel cages in a hygienic, well-ventilated room. They were fed standard rat pellets and water.

Surgical Procedures

The rats were randomized into three groups as follows: a sham group, an ischemia-reperfusion (IR) group, and an IR + CD group. In the IR + CD group, CD (Doxium; Abdi Ibrahim, Istanbul, Turkey) (100 mg/kg/day) was given to the rats for two days before laparotomy by intragastric lavage. After fasting for 12 h, the rats were anesthetized by 75 mg/kg of Ketamine (Ketalar; Parke Davis, Eczacibasi, Istanbul, Turkey) and 10 mg/kg of xylazine (Rompun; Bayer AG, Leverkusen, Germany), administered intraperitoneally. Laparotomy was then performed using a midline incision after placing the rats in the appropriate position. In the sham group, the SMA was exposed. It was dissected but not occluded. In the IR and the IR + CD groups, the SMA was exposed. It was then clamped at the level of origin from the aorta using a microvascular bulldog clamp. The clamp was removed after 60 min of ischemia, allowing reperfusion for 60 min. After the reperfusion period, 2 ml of blood were drawn from the vena cava inferior to measure oxidative stress markers. Moreover, an intestinal segment (2 cm in size) was resected from the ileum for histopathological evaluation and assessment of oxidative markers. All the rats were sacrificed under general anaesthesia.

For histopathological evaluation, approximately half of the intestinal tissue (approximately 1 cm in size) was removed and placed in a solution of 10% formaldehyde and transferred to the pathology laboratory. To determine the oxidative stress markers in the intestinal tissue, the remaining ileal segment (approximately 1 cm in size) was homogenised in ice-cold potassium chloride (150 mmol/L). The homogenates were centrifuged at 12,500 rpm, and the supernatant obtained was stored at –80° C until the assays. Blood samples drawn were centrifuged for 10 min at 3000 rpm, and the supernatant obtained was stored at -80° C until analysis of oxidative stress parameters. As the study was comparative in nature, no protective material was added to the samples.

Biochemical Analysis

Total antioxidant level (TAS)

The total antioxidant level (TAS) was measured in samples using commercial kits (Rel Assay). The measurement method relies on the proportion of the coloured radicals that undergo decolourisation relative to the total concentration of antioxidant molecules as a result of the reduction of coloured ABTS cationic radicals by overall antioxidant molecules. The results were expressed as mmol Trolox equivalent/L for serum TAS and mmol Trolox equivalent/gram protein for tissue TAS[12].

Total oxidant level (TOS)

The total oxidant level (TOS) was measured in samples using commercial kits (Rel Assay). The measurement technique is a colorimetric method, which relies on the cumulative oxidation of ferrous ion of oxidant molecules to ferric ion. Serum TOS was expressed as μmol H_2O_2 equivalent/L, and tissue TOS was expressed as μmol H_2O_2 equivalent/gram protein[13].

Oxidative Stress Index (OSI)

The OSI was defined as the ratio of TOS to TAS levels[14].

Histopathological Evaluation

After fixation in a solution of 10% formaldehyde, randomised samples were taken from the intestinal tissue specimens. The samples were embedded in paraffin blocks, and 5 μm thick sections were obtained. The samples were then stained by haematoxylin eosin for microscopic evaluation. Histopathological changes in the intestinal tissues were assessed according to the staging system proposed by Chiu et al[15].

Statistical Analysis

All statistical analyses were performed with SPSS for Windows version 11.5 (SPSS Inc.,

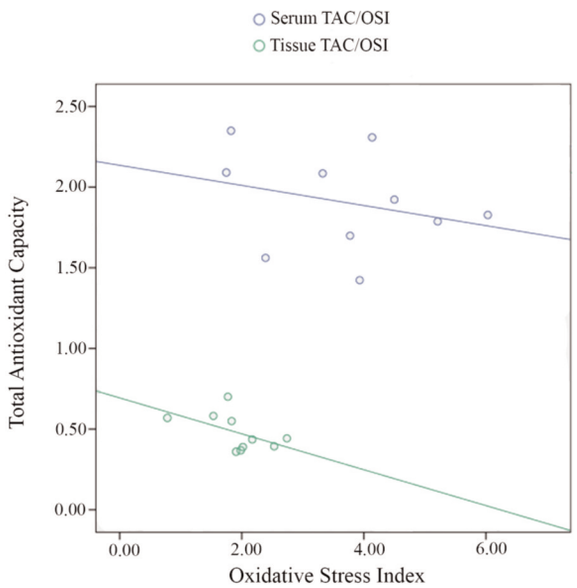

Figure 1. The relationship between serum-tissue the total antioxidant capacity and oxidative stress in the ischemia-reperfusion + calcium dobesilate group (r = –0.287, p = 0.421; r = –0.522, p = 0.122 respectively).

Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD) and categorical variables as figure and percent. The Kolmogorov-Smirnov test was used to assess the normal distribution of the data. The Levene test was used to assess homogeneity of variables. The Student's t test was used to compare parameters. A one-way analysis of variance (ANOVA) was used to compare the sham, IR and IR + CD groups. Bonferroni, Tukey and Sheffé tests were used for posthoc analysis. Fisher's exact test was used to compare categorical variables among the groups. For correlation, the Pearson correlation test was used. A two-tailed p value < 0.05 was considered significant.

Results

In the comparison of the serum and tissue oxidative stress parameters between the IR + CD and the IR groups, the OSI level was significantly lower ($p < 0.001$) and the TAS was significantly higher ($p < 0.001$) in the IR + CD group. Both serum and tissue TOS levels were lower in the IR + CD group when compared to the IR group, but the difference did not reach statistical significance ($p = 0.390$ and $p = 0.445$, respectively).

In the comparison between the IR + CD and the sham groups, the serum OSI level was lower in the IR + CD group, but the difference did not reach statistical significance ($p = 0.176$). However, the tissue OSI level was significantly lower in the IR + CD group when compared to the sham group ($p < 0.001$). The decrease in the serum and tissue OSI level was due to an increase in the TAS level (r = –0.287, $p = 0.421$; r = –0.522, p = 0.122 respectively) (Table I and Figure 1).

Table II presents the distribution of the subjects within groups according to the staging system of Chiu et al[15], and Figure 2 presents the histopathological appearance of the IRI samples. The number of subjects with stage 3 and 4 tissue injury was higher in the IR group when compared to the IR + CD group. Fisher's exact test was performed to detect whether there was a difference between the groups regarding the distribution of cases with histopathological stage 1-2 and 3-4. No significant difference was detected ($p = 0.708$ and $p = 0.667$, respectively).

Discussion

In this study, we aimed to investigate whether the antioxidant properties of CD confers protection against IRI caused by AMI, giv-

Table I. Tissue and serum oxidative stress parameters of the subjects within groups.

	Sham	IR	IR + CD	p*
Serum TAS (mmol Trolox equivalent/L)	0.95 ± 0.17	0.93 ± 0.11	1.91 ± 0.31	<0.001
Serum TOS (μmol H2O2 equivalent/L)	46.93 ± 16.73	80.83 ± 13.54	69.14 ± 26.30	0.002
Serum OSI	4.98 ± 1.74	8.79 ± 1.57	3.69 ± 1.41	<0.001
Tissue TAS (mmol Trolox equivalent/L)	0.16 ± 0.06	0.15 ± 0.05	0.48 ± 0.11	<0.001
Tissue TOS (μmol H2O2 equivalent/L)	5.33 ± 1.36	10.03 ± 2.00	8.94 ± 2.43	<0.001
Tissue OSI	4.03 ± 2.06	7.00 ± 2.23	1.93 ± 0.54	<0.001

All the data were expressed as the mean ± standard deviation, *One-way analysis of variance (ANOVA)
Abbreviations: IR: ischemia-reperfusion, IR+CD: ischemia-reperfusion + calcium dobesilate, TAS: total antioxidant level, TOS: total oxidant level, OSI: oxidative stress index

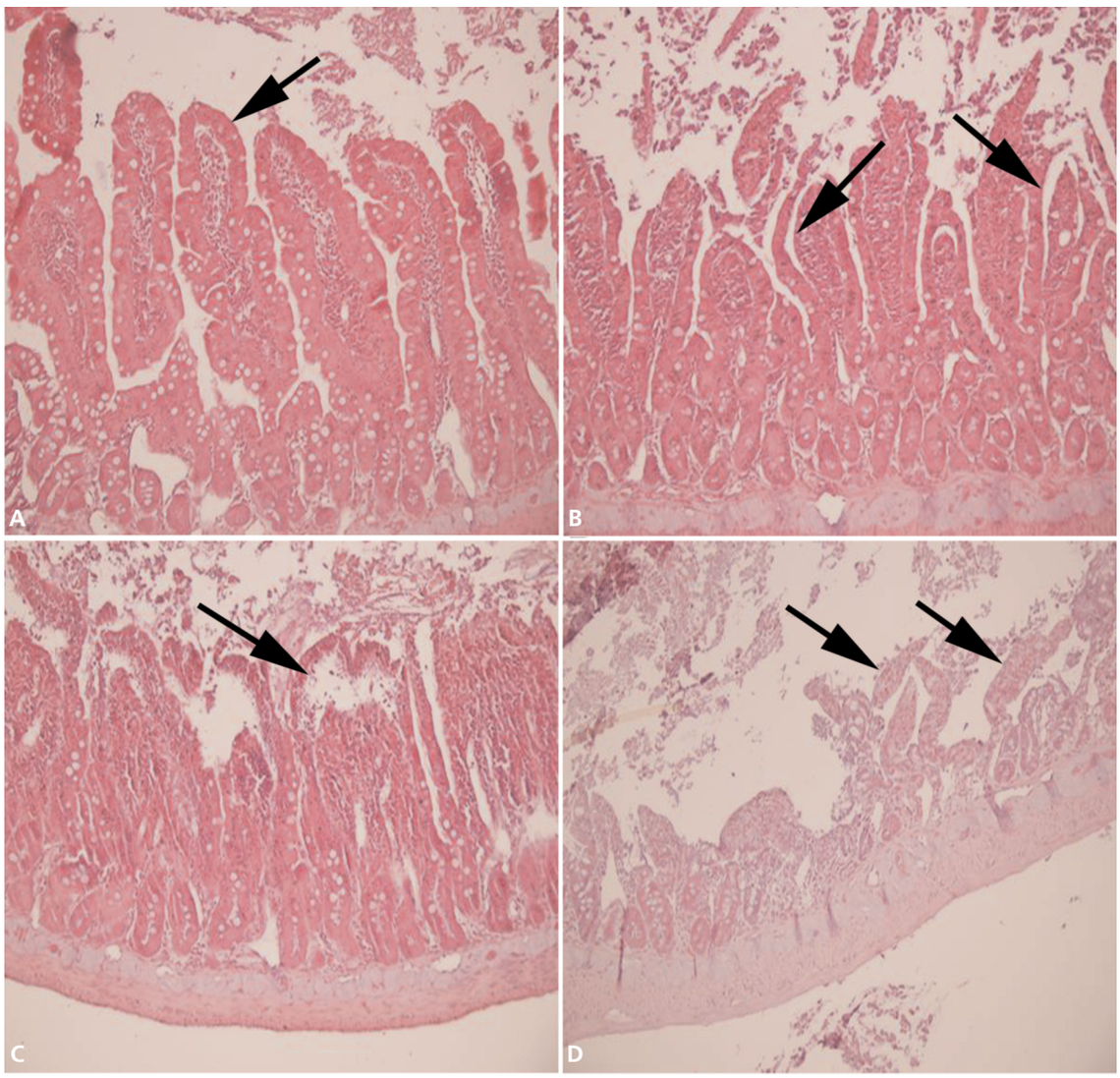

Figure 2. Histopathological appearance of ischemia-reperfusion injury according to the staging system of Chiu et al[15] (haematoxylin eosin × 200). ***A,*** Mild enlargement of subepithelial area within villi and capillary congestion in villi (stage 1). ***B,*** Moderate resolution of epithelial cells lining the villi from lamina propria (stage 2). ***C,*** Epithelial cell layer lining the villi of the lamina propria, extending to basal layer (stage 3). ***D,*** Complete strip of epithelial cell layer lining the villi and capillary vascular dilatation (stage 4).

en that AMI is associated with high mortality and that oxidative stress is implied in its pathophysiology. The primary findings of this study can be summarized as follows: (I) When compared to the IR group, both serum and tissue OSI were lower in the IR + CD group, (II) pathological injury was lower in the IR + CD group, (III) the tissue OSI in the IR + CD group was lower than that in the sham group, and (IV) the decrease in the OSI was related to increased tissue levels of TAS.

IRI is a devastating clinical condition that may result from several pathological events, such as haemorrhage, AMI, trauma and septic shock[16]. Research has suggested that ROS and endogenous cytokines, such as PAF, are involved in the development of intestinal IRI[17]. One study demonstrated that oxygen supplied during the reperfusion period increased ROS formation[5]. Another study proposed that there is increased PAF release from macrophages, mast cells and endothelial cells, which, in turn, exert a chemo-

Table II. Distribution of subjects within groups according to histopathological stages.

	Stage 0 (n = 10)	Stage 1 (n = 3)	Stage 2 (n = 7)	Stage 3 (n = 6)	Stage 4 (n = 4)
Sham	10	-	-	-	-
IR group	-	1	2	4	3
IR+CD group	-	2	5	2	1

Abbreviations: IR: ischemia-reperfusion, IR+CD: ischemia-reperfusion + calcium dobesilate.

tactic effect on leukocytes thought to mediate reperfusion injury[18].

Dobesilate is a synthetic derivative of benzene sulphonate. It exhibits antioxidant and vasoprotective activity and is widely used in the treatment of diabetic retinopathy, chronic venous failure and haemorrhoidal disease[10,19]. The antioxidant properties of dobesilate, when used in combination with calcium and magnesium ions in clinical practice, have been attributed to neutralisation of ROS and decreased release of PAF from platelets[20]. In this study, we aimed to investigate whether the neutralising of ROS and the decrease in PAF associated with CD ameliorate injury caused by intestinal IRI.

Research has suggested that hypoperfusion in the gastrointestinal system plays a role in the development of pathological conditions, such as small intestine dysmotility and mucosal barrier dysfunction[21]. In experimental models of SMA occlusion and reperfusion, it is shown that severe injury developed at the intestinal mucosa and wall[5,21]. The injury was attributed to increased ROS after reperfusion[5,6]. In our study, no histopathological injury was detected in the sham group, whereas histological injury was detected in all the rats in the other groups according to the staging system of Chiu[15] (Table II). There was less histopathological injury in the IR + CD group when compared to the IR group.

Reperfusion following tissue ischemia increases the oxygen concentration in the tissue. The increased oxygen reacts with hypoxanthine and xanthine oxidase in ischemic tissue. As a result, ROS formation is triggered[5]. Oxidative stress causing tissue and cell injury occurs if ROS is not inactivated by antioxidant metabolism. As a previous study showed a significant correlation between TOS and the OSI in the body[8], we used the OSI level to estimate the level of oxidative stress in both serum and tissue. The OSI level was lower in the IR + CD group when compared to the other two groups. The decrease in the OSI level resulted from the CD-induced increase in the antioxidant capacity of both the serum and tissue.

Conclusions

This study demonstrated that CD reduces both the level of oxidative stress and histological injury in AMI, which requires acute surgical intervention and is associated with high mortality. Thus, CD use in AMI could be beneficial to reduce mortality in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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