



Histopathological Evaluation on the Effects of Venesection and Vitamin C on Systemic Renal and Hepatic Lesions after Limb Ischemia-Reperfusion in Rabbit

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Abstract

Objective- To evaluate the effects of venesection and vitamin C on systemic renal and hepatic lesions after limb ischemia-reperfusion in rabbit.

Design- Experimental study.

Animals- 20 male white New Zealand rabbits between 2.5-3 kg, randomly assigned in 5 equal groups.

Procedures- After general anesthesia right femoral artery and vein were approached from inguinal region. In treatment and control groups the ischemia was induced by occlusion of both vessels for six hours, using a Rumel tourniquet. The same procedures were performed for sham groups, except the occluding of the vessels. In treatment1 and sham1 groups, 500 mg/kg vitamin C was injected intramuscularly during 30 min before occluding of the vessels and before the end of the operation, respectively. The same volume of normal saline was injected in control and sham 2 groups. For animals in the treatment 2 group, at the end of the ischemic period, the arterial tourniquet was released to allow arterial inflow before commencement of venous aspiration. The venous blood was aspirated into a syringe (0.5% of the animals' body weight). After 72 hours, the animals were sacrificed and tissue samples were harvested from the kidney and the liver, to assess for histological evidence of injury in these organs.

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Results- Limb I/R injury resulted in glomerular and epithelial alterations in the kidney, and also necrosis, capillarization, biliary hyperplasia, thrombosis and hemorrhage in the liver. Minimal microscopic renal and hepatic changes were seen in ischemic rats which treated by vitamin C.

Conclusion and Clinical Relevance- This study showed that venesection was not successful in preventing kidney and liver from destruction. However, vitamin C therapy protected both organs against acute and lethal lesions due to limb I/R injury.

Key Words- ischemia/reperfusion (I/R) injury, venesection, vitamin C, limb, kidney, liver

Introduction

Various mechanisms have been implicated to explain the development of ischemia/reperfusion (I/R) injuries in body organs. Stimulated generation of superoxide (O_2^-) and reduction of nitric oxide (NO) production are believed to play a key role in this process. Superoxide induces generation of other extremely reactive oxygen-derived free radicals (OFR). Excessive OFR formation overwhelms the endogenous antioxidative defense capacity, resulting in cell damage through increased lipid peroxidation in cell membranes. The consequences of such injury are remote and local tissue destruction. Deficiency of vasodilator NO due to its consumption by vasoconstrictor OFR results in microvascular constriction and prominent reduction of blood flow in reperfused tissue. Oxygen-derived free radicals also promote the formation of inflammatory responses causing leukocyte "rolling and sticking". In addition, altered rheological conditions lead to thrombus formation in microvessels. These mechanisms contribute to the development of no-reflow phenomenon.¹

Different treatment strategies are available for reducing I/R injury. These therapies include, supplementation of NO, vitamins, hydrolytic enzymes, herbal remedies, and surgical techniques e.g venesection.^{1,2,3,4,5} However, there is not enough comparative investigation on venesection and chemical therapies. In this investigation, two methods of surgical (venesection) and chemical treatments are used and compared to protect two remote organs (kidney and liver) against the effect of limb I/R injury.

Materials and Methods

Animal grouping

The study was performed on 20 male white New Zealand rabbits weighing 2.5-3 kg, randomly assigned in 5 equal groups.

Group A (Treatment1): administration of vitamin C, 6 h. ischemia, 72 hours reperfusion.

Group B (Treatment2): 6 h. ischemia, venesection, 72 hours reperfusion.

Group C (Control): administration of normal saline, 6 h. ischemia, 72 hours reperfusion.

Group D (Sham 1): administration of vitamin C, the same procedure as group A without vessel occlusion.

Group E (Sham 2): administration of normal saline, the same procedure as group C without vessel occlusion.

Surgical procedures

The operation was performed under general anesthesia using halothane/oxygen, delivered from an anesthetic machine through a face mask. The animal was positioned dorsally and the

right inguinal region and proximal medial thigh were prepared for aseptic surgery. Right femoral artery and vein were approached from inguinal region. In treatment and control groups the ischemia was induced by occlusion of both vessels for six hours, using a Rumel tourniquet. The surgical field was covered by saline soaked gauze and sterile drape until the end of ischemia period, when the tourniquet was released and the wound was closed routinely.

The same procedures were performed for sham groups, except the occluding of the vessels. In treatment1 and sham1 groups, 500 mg/kg vitamin C was injected intramuscularly, and slowly from 30 min before occluding of the vessels and at the end of the operation, respectively. The same volume of normal saline was injected in control and sham 2 groups.

For animals in the treatment 2 group, at the end of the ischemic period and prior to arterial tourniquet release, the adjacent femoral vein was isolated and occluded using vessel loop wound around the vein in a figure-of-8 fashion. Then, the arterial tourniquet was released to allow arterial inflow before commencement of venous aspiration. The venous blood was aspirated into a syringe (0.5% of the animals' body weight). Following venesection, the vessel loop was removed and hemostasis was achieved with digital pressure.

After 72 hours, the animals were euthanised by intracardiac injection of 10 mg/kg Na thiopental and tissue samples were harvested from the kidney and the liver, to assess for histological evidence of injury in these organs⁵. All the procedures were conducted in accordance with the European community guidelines for laboratory animals.

Histopathological examination: Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thickness were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope.

Results

Macroscopic findings

The kidneys were much darker red in groups B (treatment2) and C (control) , compared with the other groups.

The livers in groups B (treatment2) and C (control) showed areas of paleness and hemorrhage, intermittently. The livers in groups D (sham 1) and E (sham 2) were nearly normal, and the liver in group A (treatment1) showed mild hyperemia.

Microscopic findings:

Renal and hepatic histopathological changes are shown in Tables 1 and 2, respectively (Figs 1-4).

Table1: Renal histopathological changes due to venesection and vitamin C administration after limb ischemia-reperfusion in rabbit.

Lesion Group	Hyperemia & Hemorrhage	Epithelial necrosis	Glomerular atrophy	Tubular dilatation	Cellular cast
Group A (Treatment1)	- ^a	+ ^b	-	-	-
Group B (Treatment2)	+	++ ^c	+	+	+
Group C (Control)	+	++	+	+	+
Group D (Sham 1)	-	-	-	-	-
Group E (Sham 2)	-	-	-	-	-

^a: no lesion ^b: mild ^c: severe

Table 2: Hepatic histopathological changes due to venesection and vitamin C administration after limb ischemia-reperfusion in rabbit.

Lesion Group	Hemorrhage & Thrombosis	Biliary hyperplasia	Centrilobular degeneration & Necrosis	Capillarization	Dilatation of the central vein	Hyperemia
Group A (Treatment1)	- ^a	+ ^b	-	+	+	+
Group B (Treatment2)	++ ^c	++	+	++	+	+
Group C (Control)	++	++	+	++	+	+
Group D (Sham 1)	-	-	-	-	-	-
Group E (Sham 2)	-	-	-	-	-	-

^a: no lesion ^b: mild ^c: severe

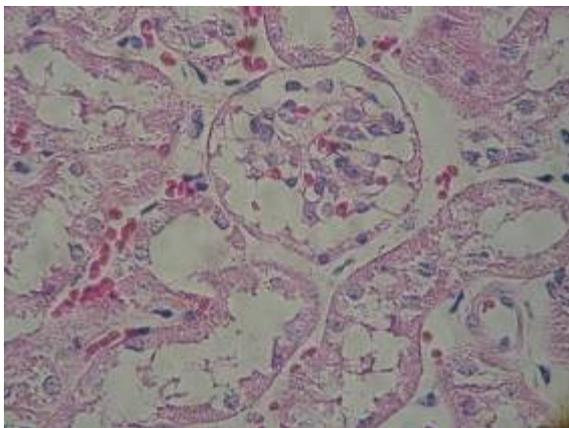


Figure 1. Renal changes due to I/R injury. Hyperemia, hemorrhage, epithelial and glomerular changes are seen. (200×,H&E)

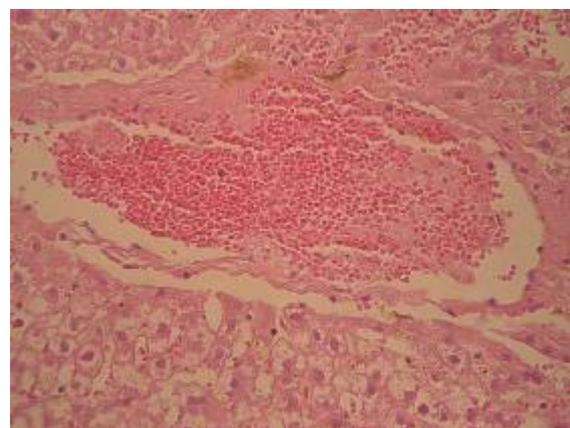


Figure 2. Hepatic changes due to I/R injury. Note to the dilatation of the central vein and centrilobular degeneration and necrosis. (200×,H&E)

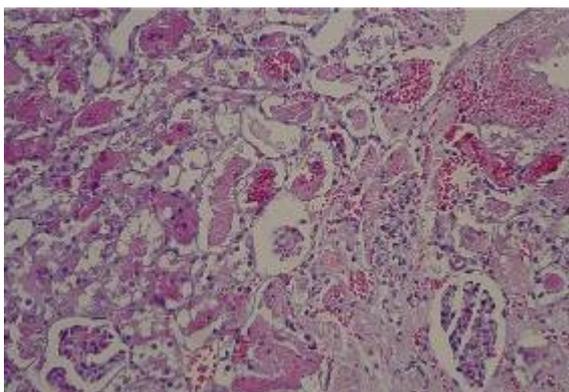


Figure 3. Renal changes due to I/R injury. Hemorrhage and epithelial necrosis are observed. (100×,H&E)

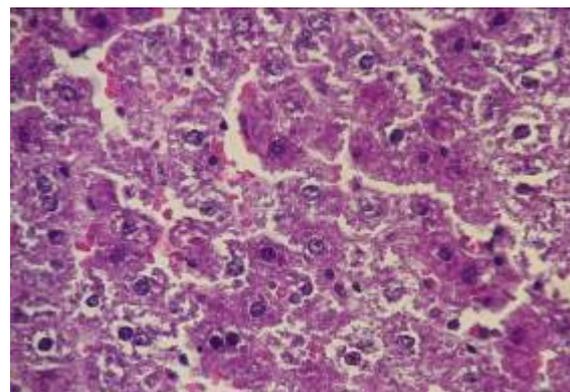


Figure 4. Hepatic changes due to I/R injury. Note to the disorganized hepatocytes with vacuolated/acidophilic cytoplasm and some pyknotic nuclei. (400×,H&E)

Discussion

Morbidity and mortality after revascularization of acute ischemic limbs remain high, despite many surgical improvements during the last decades. To a large extent, this is related to reperfusion itself after complete, acute, prolonged ischemia that results in postreperfusion syndrome.⁶ Acute limb ischemia is a common medical condition resulting from arterial embolization, in situ thrombosis, trauma, and other causes. The severity of injury is related to the duration of ischemia and the effects of reperfusion. Metabolic consequences of reperfusion injury can be variable, ranging from transient symptoms in the lower extremity to systemic inflammation with multiple organ dysfunction.⁷ Restoration of blood flow to an acute ischemic extremity may deteriorate the ischemic injury, leading to multiple organ dysfunction or even death. This paradox of continuing injury during reperfusion is not completely understood. The role of multi-organ damage in the mortality caused by ischemic limb injury is also still not clarified. Histologic examinations showed that the major systemic manifestation was massive destruction of the liver and kidney. Some researchers believe that the ratio of CK-MB isoenzyme is most useful for distinguishing the risk of mortality caused by acute ischemic limb injury, and the cause of systemic complications are attributed to the multi-organ failure.⁸

In the present study, as shown in result section, limb I/R injury resulted in glomerular and epithelial alterations in the kidney, and also necrosis, capillarization, biliary hyperplasia, thrombosis and hemorrhage in the liver. Obviously, liver destruction was more life threatening than the kidney changes. In addition, in contrast to Ho et al (2009)⁵, venesection could not prevent kidney and liver from destruction; whereas, vitamin C therapy, as some previous reports protected both organs against acute and lethal lesions due to the limb I/R injury.^{9,10}

Reperfusion of acutely ischaemic tissue may, paradoxically, lead to systemic complications. This phenomenon is believed to be initiated by humoral factors that have accumulated in the ischaemic tissue. The ancient art of venesection may reduce the load of these mediators at the point of reperfusion.⁵ Recent evidence has shown that immune phenomena are clearly related to the I/R injury, which initially activates an innate immune response through Toll-like receptors followed by adaptive immune responses. Furthermore, in knock-out mice for T or B lymphocytes or administration of depleting antibodies have shown that classical immune cells are actively involved in an I/R injury. Dendritic cells (DCs), which are usually seen to trigger the immune response by their antigen-presenting capabilities, are also involved in I/R injury. Activation and maturation of resident DCs are probably the first signals that initiate the innate immune response.¹¹ Several studies have demonstrated that reactive oxygen species (ROS) have an important role in I/R injury, especially through lipid peroxidation. They can lead to cellular injury by attacking membranes through peroxidation of polyunsaturated fatty acids, which can alter both membrane structure and function of mitochondrial, lysosomal, and plasma membranes. Cellular defense against oxidative injury is provided by several mechanisms. Antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), as well as nonenzymatic compounds such as reduced glutathione (GSH), ascorbic acid (AA), and α -tocopherol all help to cope with potential damage. Glutathione is present in all mammalian cells, especially in renal cells, hepatocytes, and erythrocytes. The increased production of ROS during I/R injury results in consumption and depletion of endogenous antioxidants. In this situation, the cells require exogenous antioxidant to protect them from ROS-induced damage.¹²

Vitamin C acts by scavenging reactive oxygen species via rapid aqueous-phase electron transfer, thereby reducing adhesion of neutrophils to endothelium. It decreases both the generation of oxygen free radicals by neutrophils and subsequent lipid peroxidation. The systemic nature of the activity of vitamin C is shown by the reduction in circulating neutrophil respiratory burst after five days of oral treatment. By lowering the proinflammatory activity of neutrophils distant to the site of injury, pretreatment with vitamin C may decrease subsequent tissue damage when these neutrophils are chemoattracted to the site of I/R injury⁹. Ascorbic acid has been used to protect against corneal damage by free radicals in rabbits. In addition, it has also been used to improve the renal hemodynamics as well as decreased oxidative stress, inflammation and fibrosis in the ischemic kidney of the pigs. Ascorbic acid is an inexpensive low-priced antioxidant that can be administered easily as it is water-soluble¹⁰. In conclusion, vitamin C as one of the most accessible drugs is recommended for I/R injury situations.

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بررسی هیستوپاتولوژیک اثرات خونگیری سیاهرگی و ویتامین ث بر ضایعات کلیوی و کبدی ناشی از ایسکمی-دوباره خونرسانی در پای خرگوش

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هدف- بررسی اثرات خونگیری سیاهرگی و ویتامین ث بر ضایعات کلیوی و کبدی ناشی از ایسکمی-دوباره خونرسانی در پای خرگوش.

طرح مطالعه - مطالعه تجربی.

حیوانات- ۲۰ سر خرگوش نر سفید نیوزیلندی با وزن تقریبی ۲/۵ کیلوگرم، به طور اتفاقی، به ۵ گروه مساوی تقسیم شدند. **روش کار-** پس از القاء بیهوشی عمومی، سرخرگ و سیاهرگ رانی سمت راست از ناحیه مغابنی در دسترس قرار گرفتند. در گروه‌های درمان و کنترل، ایسکمی توسط تورنیکت و با انسداد هر دو رگ به مدت ۶ ساعت برقرار شد. همین روش در گروه‌های شم به کار گرفته شد، اما انسداد رگ‌ها صورت نپذیرفت. در گروه‌های درمان ۱ و شم ۱، ویتامین ث به میزان ۵۰۰ میلی گرم به ازاء وزن بدن از طریق داخل عضلانی تزریق گردید، با این تفاوت که در گروه درمان ۱ تزریق در طول ۳۰ دقیقه قبل از بستن رگ‌ها و در گروه شم ۱ در طول ۳۰ دقیقه قبل از پایان عملیات انجام شد. حجم مشابهی از نرمال سالین به خرگوش‌های گروه کنترل و شم ۲ تزریق گردید. در گروه درمان ۲، با پایان گرفتن زمان ایسکمی، تورنیکت برداشته شد تا جریان خون برقرار شود و در همان حال به میزان ۰/۵ درصد وزن بدن از سیاهرگ مربوطه خونگیری بعمل آمد. پس از ۷۲ ساعت، حیوانات کشته شدند و نمونه‌های کبد و کلیه جهت مطالعات بافت شناسی به آزمایشگاه ارسال شدند.

نتایج- جراحات ناشی از ایسکمی-دوباره خونرسانی به تغییراتی در بافت پوششی و گلومرول‌های کلیه منجر گردید. علاوه بر این، نکروز، مویرگی شدن سینوزوئیدها، هیپرپلازی مجاری صفراوی، ترومبوز و خونریزی در کبد حادث گشت. از سوی دیگر، تغییرات اندک میکروسکوپی در کلیه و کبد خرگوش‌های درمان شده با ویتامین ث ملاحظه گردید.

نتیجه گیری- این مطالعه نشان داد که خونگیری سیاهرگی در ممانعت از تخریب‌های کلیوی و کبدی مؤثر نبوده است. از سوی دیگر، درمان با ویتامین ث هر دو اندام را در قبال ضایعات حاد و کشنده ناشی از ایسکمی-دوباره خونرسانی محافظت نموده است.

کلید واژگان- جراحات ناشی از ایسکمی-دوباره خونرسانی، خونگیری سیاهرگی، ویتامین ث، پا، کلیه، کبد.