Original Article

STUDY OF THE EFFECT OF STREPTOKINASE AND ALLOPURINOL ON ISLAND SKIN FLAPS SUBMITTED TO PROLONGED ISCHEMIA - EXPERIMENTAL STUDY IN RATS*

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ABSTRACT

OBJECTIVE. To establish a relation between the survival rate for island skin flaps submitted to prolonged ischemia and the effect of streptokinase and allopurinol administered following the prolonged ischemic period.

METHODS. A total of 48 male Wistar rats, each weighing between 300 and 350 grams, were separated into four groups of twelve as follows: control, allopurinol, streptokinase, and association of allopurinol and streptokinase. The rats were submitted to epigastric island flap dissection, followed by epigastric vessel bundle clamping. Flaps remained in this condition for 8 hours, in normothermic global ischemia. After the ischemic period, the clamps were removed and each rat received the proposed therapeutic scheme for the group by intravenous injections. Flap survival analysis was performed on the seventh postoperative day. Variance and descriptive analyses (as percentage of necrotic area) were carried out, as well as Dunnett's T3 multiple comparisons among the four groups and the median test.

RESULTS. Rats in the control group presented an average necrosis of 79.88% in the total flap area; those receiving allopurinol presented an average necrosis of 64.05%, whereas the group receiving streptokinase presented an average necrosis of 55.52%. With the association of both drugs, rats presented an average necrosis of 54.30% in the total flap area. By applying the Dunnett's test and the median test, we were able to verify that, in this study, the streptokinase group had the lowest rate of necrosis.

CONCLUSION. Compared to the administration of allopurinol, association of allopurinol and streptokinase, and the control group, systemic administration of streptokinase after 8 hours of normothermic global ischemia resulted in an increased survival rate for epigastric island skin flaps in rats.

KEYWORDS: Streptokinase. Allopurinol. Reperfusion injury. Surgical flaps. Free radicals.

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INTRODUCTION

The mechanisms responsible for cellular necrosis secondary to prolonged ischemia followed by reperfusion, i.e., ischemia-reperfusion injury,[1-8] have become a major scientific challenge, given their implications for trauma, organ and tissue transplants, acute myocardial infarctions, vascular injuries, and reconstructive plastic surgery.

Ischemia-reperfusion injuries are caused by microcirculatory changes, through the release of proteolytic enzymes, production of free radicals, and physical microcirculatory obstruction at the capillary level.

Therapeutic schemes are suggested to improve the results of organ and tissue transplants, microsurgical reconstructions and myocardial infarctions.[9] Secondary ischemia in transplant organs or tissues often goes undetected in the initial stages, and becomes clinically apparent only when the chance of recovering the tissue is gone, despite review of anastomoses and monitoring vascular permeability. Tissue is then not perfused, leading to necrosis. The phenomenon is known nonperfusion, due to microcirculatory thrombosis and not to pedicle thromobosis.[30,31]

The epigastric flap was chosen for this study because it has axial blood irrigation through the inferior surface epigastric vessels, easily accessible, as described by Sasaki and Pang, a model widely used in scientific research[10-15,37,39-43] on the subject of ischemia-reperfusion.

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The objective of this study was to establish a relation between the survival rate for island skin flaps in rats submitted to prolonged ischemia, and the effect of streptokinase and allopurinol, in isolation or in association, administered eight hours following ischemia, with possible prevention of the deleterious effects from the ischemia-reperfusion period.

Methods

A total of 48 male Wistar rats, each weighing between 300 and 350 grams, were separated into four groups:

GROUP 1: occlusion of inferior epigastric vessels for eight hours, followed by injection of 1 mL of sodium chloride 0.9%.

GROUP 2: occlusion of inferior epigastric vessels for eight hours, followed by injection of 1 mL of allopurinol (Sigma-Aldrich A8003), 100 mg/kg, diluted in 1 N of NaOH, tittered with 2 N of HCl to the point of crystallization.

GROUP 3: occlusion of inferior epigastric vessels for eight hours, followed by injection of 1 mL of a solution containing 10,000 international units of streptokinase (Streptase 1500000 IU -Hoeschst Marion Roussel) diluted in saline solution 0.9%.

GROUP 4: occlusion of inferior epigastric vessels for eight hours, followed by injection of 1 mL of a solution of both drugs, at the doses established above.

Surgical procedures began with anesthetizing the animals by intraperitoneal injection of pentobarbital (35 mg/kg), followed by trichotomy of the right inguinal and abdominal regions. Flap borders of 3.0 by 6.0 cm were marked on the animal's skin from the median line (left lateral limit) and right inguinal region (lower limit). To inject the drugs, rats were submitted to local anesthesia, xylocaine 2% on the manipulation site, and inhalation of diethyl ether. Analgesia during the skin flap's ischemic period was produced using intramuscular dipyrone (30 mg/kg).

The incision was made on the region from the borders marked on the skin to the aponeurotic plane, except for the inguinal region, where only the skin was incised, followed by dissection of the skin flap on the cranial-caudal direction, with clear sight of the vascular-nervous pedicle, making the procedure safer[16] (Figure 1). The vascular-nervous bundle was dissected with the aid of a microsurgical microscope, followed by clamping the vessels with microsurgical clamps.

A continuous suture was made using monofilament 5-0 nylon threads from the skin flap to its bed, except at the lower right end of the flap, where suture was made using simple stitches. Animals were once again anesthetized, and microsurgical clamps were removed after eight hours of normothermic and global (arterial and venous) ischemia through the lower right end of the flap. After clamps were removed, an incision was made on the left inguinal region and, with the aid of a microsurgical microscope, and the left femoral vein was catheterized using an 0.7 mm silicon catheter to administer the corresponding therapeutic scheme of each group.

The animals were kept in individual cages, receiving water and ration ad libitum, under daily observation until the seventh day, when they were again anesthetized, tied to the board for graphic transposition of viable and necrotic areas (Figure 2) from skin flaps to transparent acetate sheets laid over the flaps. The images were analyzed by transposition of necrosis and total areas to acetate paper and comparison between areas. The animals were sacrificed using anesthetic gas, as per the guidelines of the American Veterinary Medical Association and the Federal Board of Veterinary Medicine.

Variance and descriptive analyses were carried out, as well as Dunnett's T3 multiple comparisons among the four groups.

Figura 1 - Falta legenda em inglês

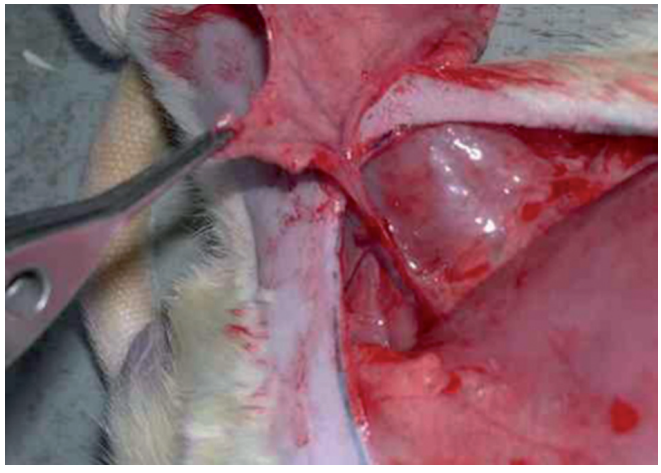
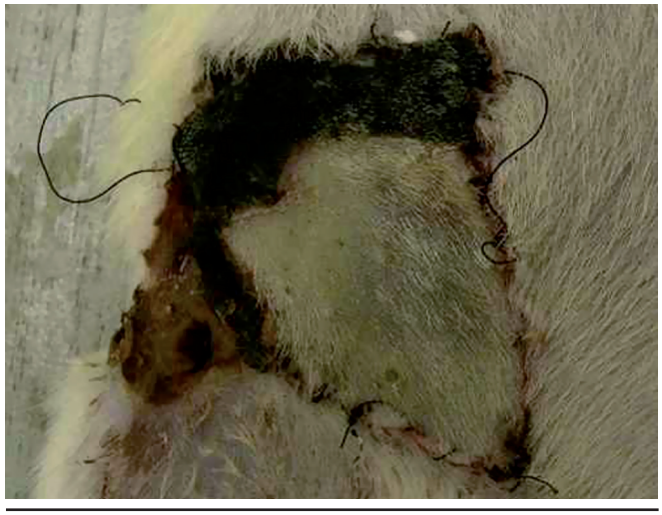


Figura 2 - Falta legenda em inglês



Results

Analyzing the skin flaps after seven days, animals from the control group presented an average necrosis of

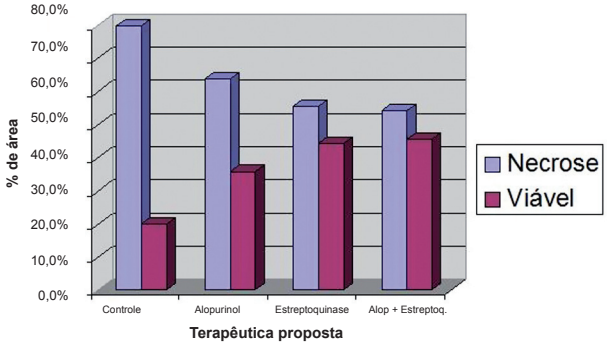
79.88% of the total epigastric flap area; those receiving allopurinol presented an average of 64.05%, and the group receiving streptokinase presented an average necrosis of 55.52% of the skin flap. With the association of both drugs, rats presented an average necrosis of 54.30% of the total flap area.

Figure 3 lists descriptive measures. Notice that the streptokinase group has the lowest standard deviation of all, making it the group with the least disperse outcomes, an average similar to the allopurinol-streptokinase group.

Applying Dunnett's test, we find that the streptokinase group is unlike the control group ($p = 0.008$), i.e., the streptokinase group presents higher concentration of measures near the average, and those are the lowest in the study.

Applying the median test, we find that the streptokinase group presented only two measures above the median (overall), versus nine measures in the control group, i.e., there are fewer measures greater than the median in this group than in others. The streptokinase group presented the lowest measures, i.e., the lowest rate of necrosis in this study.

Figura 3 - Falta legenda em inglês



Discussion

Understanding the events responsible for ischemia-reperfusion injuries helps us achieve better results in reconstructive surgeries, minimizing surgical morbidity.

In skin flap cases, after dissection, a hyperadrenergic state is seen secondary to sectioning the sympathetic nervous system's nerve endings on the borders of the skin flap. At the same time, neurotransmitters such as norepinephrine build up, leading to vasoconstriction, which in turn leads to full temporary occlusion of the capillaries. The skin flap is then already submitted to some degree of ischemia. When neurotransmitter activity ceases, the capillaries become dilated, allowing reperfusion; dilation may last from 8 to 30 hours.[11]

In instances of pedicle thrombosis, there are quick increases in intraluminal pressure, followed by interstitial edema,[15] which acts as a barrier for oxygen diffusion, causing injuries to endothelial cells and the destruction of intercellular bonds, exposing the subendothelial matrix. Platelets adhere to the subendothelial matrix mediated by collagen, fibronectin and Von Willebrand factor.[17-20]

Activated platelets degranulate, releasing ADP and serotonine, which induce the adhesion of other platelets. Thrombin is released, leading to the conversion of fibrinogen into thrombus.

Also, injured endothelial cells lose their antithrombotic and fibrinolytic properties, ceasing to secrete prostacyclin, a powerful vasodilatory and anti-aggregatory agent for platelets. This causes a state of local hypercoagulability, contributing to microcirculatory thrombosis.

Ischemia leads to the exhaustion of ATP reserves. With the accumulation of hypoxanthine, there is irreversible conversion of the physiological form of the xanthine dehydrogenase enzyme into xanthine oxidase; the substances build up while the tissue remains submitted to ischemia.[21,22]

In every state of hypoperfusion, these mechanisms are normally activated and in equilibrium with chelating mechanisms of free radicals, such as endogeneous enzymes, superoxide dismutase, catalase, and glutathione peroxidase.

Xanthine oxidase converts hypoxanthine and xanthine into uric acid, using the oxygen entering the tissue during reperfusion as cofactor. This reaction leads to the release of free radicals derived from oxygen.[23-26] These molecules have an unpaired electron in their outer orbital, making them highly reactive. The radicals, peroxide, superoxide, and hydroxyl, destroy the integrity of microvascular architecture by lipid peroxidation of compounds of the cell membrane and organelles; they also injure the endothelial basement membrane through hyaluronan disaggregation.[27]

Ischemia also leads to increased expression of ICAM-2, the molecules responsible for leukocyte adhesion to the endothelium.[5,28,29]

Hypercoagulability, free radical injuries, leukocyte adhesion, interstitial edema, vasospasm, and stasis events lead to irreversible microcirculatory thrombosis. The sooner this cascade is reversed, the greater the chance for tissue viability, since these are time-dependent phenomena.[12]

In this context, some drugs are being used to attempt to minimize and prevent such events. This study sought to analyze the effects of streptokinase (a thrombolytic) and allopurinol (a xanthine oxidase inhibitor) in skin flaps submitted to prolonged ischemia.

It is believed that normothermic venous ischemia for eight hours leads to irreversible necrosis in epigastric skin flaps in rats,[12] though there are exceptions in similar experimental models.[32] Global ischemia (venous and arterial), on the other hand, is more easily tolerated. Low tolerance to venous ischemia is primarily due to progressive increases in intraluminal blood pressure, which accelerates the pathological processes of the tissue injury, such as abrupt endothelial edema with hindered gas diffusion.

Thus we were able to determine the 8-hour period as prolonged ischemia, and administer drugs at the moment of reperfusion, in an attempt to mimic cases such as amputations, serious trauma, vascular thromboses, and microsurgical flaps.

Increased survival rates for skin flaps to which streptokinase was administered presented a significant statistical difference in this series. On the other hand, the use of allopurinol and the association of both drugs did not present increased survival rates for skin flaps.

Conclusion

Several studies have proved the beneficial effects of using allopurinol as pretreatment, i.e., moments before raising the skin flap, or before reperfusion.[10,11,13,33] Increased stress on the scar from greater migration of fibroblasts in ischemic injuries has also been found in rats[34] treated with allopurinol. The benefits would probably be even higher if allopurinol was administered as pretreatment, preventing the formation of free radicals, since they inhibit xanthine oxidase instead of acting directly on free radicals.

Notice that studies using pigs did not find any significant statistical differences when using allopurinol, which might be explained by the fact that xanthine oxidase levels are 40 times higher in the skin of rats than in that of pigs and humans.[21,35]

Streptokinase, accepted in clinical practice as a therapy for myocardial infarctions, among other uses, on the other hand, seems to have the greatest effect when used as a vehicle for unblocking skin flap vessels, with the thrombolytic solution under greater pressure than the animal's in the attempt to revert processes leading to nonreperfusion.36,37,38

Once microcirculatory thrombosis is established, the animal's blood pressure does not seem to be enough to take the drugs to the ischemic tissue, which might have been the cause of the small improvement when the thrombolytic was used in this series.

Research using other therapeutic schemes, as well as different ischemic periods and tissues, are needed to understand and minimize ischemia-reperfusion injuries, obtaining better surgical outcomes, thus achieving better prognoses for patients suffering from this kind of injury.

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