Acute Remote Preconditioning Augments Random Skin Flap Survival, But Not Recipient-Bed Isolated Flaps In Rats

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Citation

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Abstract

This study proposes that acute remote preconditioning (ARIP) can effectively enhance random skin flap survival.The study was a randomized controlled trial using male sprague-Dawley rats as subjects. For acute remote preconditioning, left hindlimb ischemia was achieved by clamping the femoral artery and vein.After 1 hour's ischemia, the limb reperfusion was done for 30 minutes. Then, a 3- by 9- cm dorsal caudal-based, random pattern skin flap was elevated and reaproximated for flap survival studies. Thirty rats were divided into three groups of 10 rats each. The first group had only the flap raised, while the second and third groups had acute remote preconditioning protocol before the flap elevation. In the third group, a silicone sheet was inserted beneath the flap in order to prevent neovascularisation from the bed. The amount of flap necrosis was measured on the seventh postoperative day. ARIP (acute remote ischemic preconditioning) group had the most improved skin flap survival rate, and the flap survival rates between the ARIP+silicone sheet and control groups was not statistically different ($p>0.05$). These findings indicate that remote ischemic precontioning enhances random skin flap survival, when it is performed just before the flap harvest and the isolation of recipient bed abolishes this ameloriating effect.

INTRODUCTION

Allow for protection of briefly ischemic tissues against the harmful effects of subsequent prolonged ischemia is a phenomennon called as Ischemic Preconditioning (IP). Adaptational responses to Ischemia/Reperfusion (I/R) injury have been demonstrated in different tissue types.There are two distinct types of protection afforded by this adaptational reponse, i.e. acute and delayed preconditioning. The factors that initiate the acute and delayed preconditioning responses appear to be similiar. However, the protective effects of acute preconditioning are protein synthesis independent, while the effects of delayed preconditioning require protein synthesis ([1]). The inflammatory mediators released as a consequence of reperfusion also appear to activate endothelial cells in remote organs that are not exposed to the initial ischemic insult.This second phenomennon is called as acute remote ischemic preconditioning (ARIP). ARIP has been reported to be succesful for organs such as the heart, kidney and liver ([1]). Verdouw et al. were the first to report a real remote ischemic preconditioning of the heart by mesenteric artery occlusion in a rat model ([2]). The protective influence of limb ischemia on myocardial infarction was reported by Birnbaum et al. and Oxman et al. ([3,4]). Kuntscher et al. showed that ischemic preconditioning and enhancement of flap survival can be achieved not only by preclamping of the flap pedicle, but also by induction of an ischemia/reperfusion event in a body area distant from the flap before harvest ([5]). The exact mechanism of classic and remote preconditioning has not yet been determined. We aimed to evaluate the effects of ARIP on a different random pattern skin flap model with a different model of limb I/R protocol and the impact of recipient-bed isolation on skin flap survival with remote protection by ischemic preconditioning.

MATERIALS AND METHODS

Thirty male Wistar rats weighing between 230 and 335 g were divided into three experimental groups. In the control group (n = 10), caudal-based random skin flaps in adiameter of 3x9 cm were elevated and resutured the original bed with continous subcutaneous 4/0 prolene sutures.In the ARIP group, the same random skin flap elevated just after the completion of 1 hour's ischemia and 30 minutes reperfusion of the left hindlimb.In the ARIP+silicone sheet group, the flap was thereafter sutured back and placed onto a silicone sheet to prevent neovascularization from the wound bed.The flap site was prepared by shaving with electric hair clippers and betadine.Ischemia of the left hindlimb was induced by clamping left femoral artery and vein using Acland V3

clamps.Following ischemia period, the clamps were removed, and the return of vascular flow was confirmed by observation of the artery and vein through the microscope. No significant vasospasm was observed and no vasodilators were employed. On the fifth and nineth postoperative day all the animals were anesthesized and photographised. On the seventh postoperative day, total area of the flap and the area of flap necrosis were measured. First, it was drawn on acetate paper while the rats were anaesthetised, and the areas drawn were calculated in mm^2 . All experiments was approved by Institutional Committe of Animal Care and Use.

RESULTS

No complications such as hematoma, infection or destruction of suture line developed. No animal died due to the surgical procedure in any group. The necrosis become evident between the second and fourth days starting at the distal part of the flap and was well demarcated at the end of a week.Table 1 shows the results of flap survival rates in groups. ARIP group had the most improved skin flap survival rates (Fig 1 and 2). The difference between the groups of ARIP+silicone sheet and control was not statistically significant (p=0,226).

Figure 1

Table 1: Comparision of flap necrosis in the three study groups (no=10 in each group) Compared with control ; ** p =0.519 and *** p

Variable Mean (SEM)	Control Mean(SEM)	ARIP Mean (SEM)) p*value	ARIP + silicone sheet Mean (SEM) p* value
Weight (g)	230.69 (3.3)	228.40 (3.7) ** 0.849	235.10 (4.1) 0.67
Percentage of Flap necrosed	38.7 (0.4)	9.3 (1.3) *** <0.0001	34.1 (3.3) 0.226*

Figure 2

Figure 1a : Usual skin necrosis patterns in ARIP group

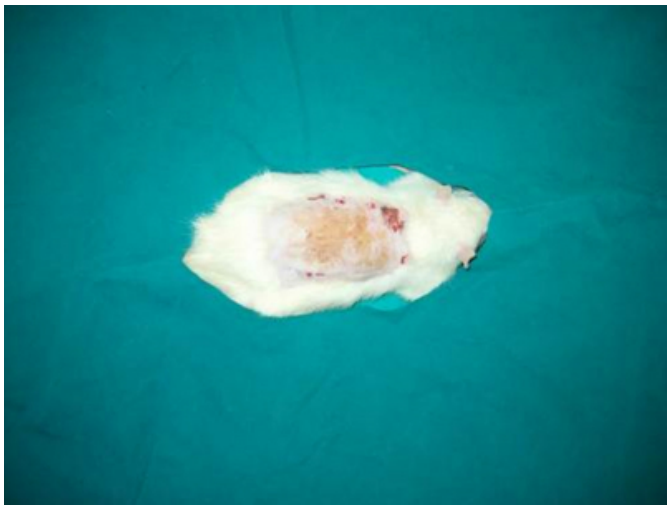
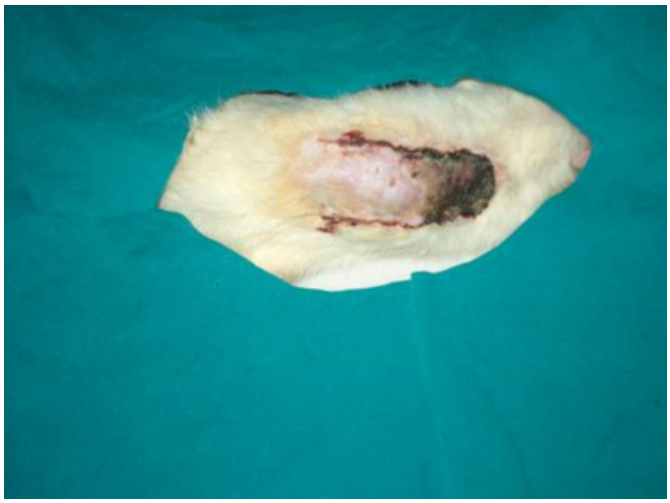


Figure 3

Figure 1b : Usual skin necrosis patterns in control and ARIP+silicone sheet groups.



STATISTICAL ANALYSIS

The difference in the mean area of necrosis and mean percentage of flap necrosis between the three groups were analysed using Kruskal-Wallis one-way analysis of variance. The difference in the mean area of necrosis and mean percentage of flap necrosis between the two individual groups were analysed using Mann-Whitney U-test. Probabilities of less than 0.05 were accepted as significant.

DISSCUSSION

Flap design is important in decreasing the risk of partial necrosis in pedicle flaps ([6]). The inclusion of a spesific vascular supply or an anatomic component within a skin flap can significantly improve survival lenght ([7]). Mc Farlane et

al in 1965 originally described the dorsal skin flap of the rat as a random pattern flap for studying ([8]). The caudally based random flap was selected as the experimental model because its consistency and standartisation were validated by Khouri et al ([9]). Sympathetic regulation of the circulation within acutely elevated random flaps was shown to have a significant role in the maintanence of flap survival ([10]). Different mechanisms including arterial vasospasm, depletion of high energy phosphates, production of oxygen free radicals, inactivation of sodium and potassium pumps, oedema in the endothelial cells probably have a role in the pathophysiology of flap ischemia and resulting necrosis.

Ischemic preconditioning induced microcirculatory protection appears to be a systemic rather than a local phenomenon. This protection induced by remote ischemic preconditioning may be attributed to humoral rather than a neuronal mechanism.A significantly higher red blood cell velocity in the first-order arterioles and capillaries, a higher capillary flow, and a decreased number of leukocytes adhering to the endothelium of the postcapillary venules were been implicated in acute remote preconditioning using a rat cremaster flap in vivo microscopy model([11]). Ischemia of 45 min followed by 2 hr of reperfusion in the left lower extremity of the rat induced a significant microvascular protection against subsequent 4-hr ischemia in both innervated and denervated cremasters ([12]). In a modified rat epigastric skin flap model, the effect of recipient bed isolation with artificial barriers on skin flap survival were evaluated by Jones et al. They have found that neovascularisation could be prevented by recipient bed isolation with an artificial barrier and the isolation of recipient bed decreased the skin flap survival ([13]).In their experimental flap model, an extended epigastric adipocutaneous flap (6 × 10 cm) based on the left superficial epigastric artery and vein was used.The limb I/R protocol was 10 minutes ischemia followed by 30 minutes of reperfusion in that study ([5]). A large body of evidence suggests that remote preconditioning can protect the distant tissues ischemic events ([14],[15],[16]). In this study, remote preconditioning produced by femoral artery occlusion for 1 hour and followed by 30 minutes reperfusion prior to random skin flap elevation resulted an improvement in skin survival area in a rat model of caudal based random pattern flap using ARIP protocol in comparision the other groups in the study. When comparing control and ARIP+silicone sheet group, there was not an statistically significant defference. In other words, isolation of the bed with silicone sheet had a negative impact on skin flap survival. This finding was comparable with the findings of previous studies ([6],[13],[17]). Although the graft effect of the bed at the flap-bed interface is controversial, factors that trigger neovascularisation have been found to be salutary on random skin flap survival ([18],[19]). Adenosine released in increased amounts by hypoxic tissues is an potential mediator of compensatory angiogenesis with other factors that include VEGF, BFGF and IGF-1 ([20]). Adenosine treatment has been shown to augments random flap survival in rats ([21]). Adenosine is though to be an angiogeneic factor that links altered cellular metabolism caused by oxygen deprivation to compensatory angiogenesis.ARIP can be hypothesized to enhance the revascularization of the severely ischemic distal random segment of the flap.The present study showed that ARIP could augment the random pattern skin survival and this effect abolished when recipient bed was isolated. Transient limb ischemia is a simplepreconditioning stimulus with important potential clinical applications.Remote ischemic preconditioning prevents IR-induced endothelial dysfunction in humans and reduces the extent of myocardial infarction in experimental animals ([22]). Remote preconditioning is systemic phenomennon and due to humoral mechanism ([12]). Nitric oxide generation may not be involved in this humoral mechanism of remote ischemic reconditioning([23]).Activation of adenosine A1 –receptors have been shown to reduce infact size and blockade of this receptors abolished this preconditioning effect ([24]). These activated A1 –receptors can also couple to ATP-sensitive potassium ion (KATP) channels results in the shortenning of action potentials and slowing of cellular ATP catabolism, and increasing myocardial ischemic tolerance ([25]). It

was shown that simple liposomal-mediated gene transfer could result in a potentially useful biological effect in the field of wound healing injected into rat skin 1 week before raising a random pattern 3 x 10 cm flap and the flap survival was enhanced by 14 percent ([26]).

Current evidence suggests that neovascularization is mediated by a wide range of angiogenic growth factors. Insufficient angiogenesis and microcirculatory intravascular clotting have been implicated in the pathophysiology of skin flap failure.VEGF protein is signicantly increased in the skin flap with mild ischemia, but decreased in the flap with severe ischemia. Vascular endothelial growth factor (VEGF) appears to be one of the most important angiogenic factors in vivo. Histological examination revealed increased density of

the capillaries in the flaps treated with VEGF when compared to the control group[27].Delivery of the gene for VEGF have been shown to improve the survival of ischemic skin flaps[28].This study generated the observation that benefical effect of ARIP is only works in case of a well vascularised recipient bed. Further studies are planned to clearify the relation between remote preconditioning and angiogenesis.

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