Egyptian Journal of Surgery Vol 26, No 4, Oct., 2007

ORIGINAL ARTICLE

EFFECT OF ACUTE REMOTE ISCHEMIC PRECONDITIONING ON SKIN FLAP SURVIVAL: EXPERIMENTAL STUDY

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Aim: ***This experimental study is a randomized controlled trial that was designed to study the effect of acute remote ischemic preconditioning (ARIPC) on random skin flap survival in rats with and without recipient bed isolation.***
Methods: ***Thirty rats were divided into three equal groups. On the dorsal aspect of the rat a caudally based, random pattern skin flap 3x9 cm in dimensions was designed. In the first (control) group, the flap had only been elevated and repositioned in place without ARIPC. In the second group, the flap was elevated and repositioned after a protocol of ARIPC. In the third group, the same protocol of group II was followed but a silicone sheet was inserted beneath the flap to prevent neovascularization from the bed. The amount of flap necrosis was measured on the seventh postoperative day.***
Results: ***The second (ARIPC) group had the most improved skin flap survival rate (P< 0.0001) while the flap survival rate in the third and control groups was not statistically different (P>0.05).***
Conclusion: ***Acute remote ischemic preconditioning enhances random skin flap survival, when it is performed just before the flap harvest and the isolation of recipient bed abolishes this enhancing effect.***

Keywords: ***preclamping, adipocutaneous flaps, Ischemia / reperfusion.***

INTRODUCTION

Ischemic preconditioning (IPC) means protection of tissues against the harmful effects of prolonged ischemia by inducing controlled brief ischemia to these tissues. Adaptation responses to Ischemia/Reperfusion (I/R) injury by either acute or delayed preconditioning have been demonstrated in different tissue types.[1-7] 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.[8] Ischemic preconditioning (IPC) effects have been reported in these remote organs without direct ischemia of them. This second phenomenon is defined as acute remote ischemic preconditioning (ARIPC).[9] ARIPC has been reported to be successful for organs such as the heart, kidney, lung and liver.[9-12] Küntscher 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.[13] Jones et al. Found that isolation of the bed of the flap with silicone sheet had a negative impact on skin flap survival.[14] The aim of this experimental study was to evaluate the effects of ARIPC on a random pattern skin flap survival with and without recipient bed isolation.

MATERIALS AND METHODS

Thirty male Wistar rats weighing between 250 and 300 grams were divided equally into three experimental groups. There was no statistical significant difference in the mean body weight of animals in the three groups (P>0.05) Table 1. All animals were anesthetized with intraperitoneal

sodium pentothal in a dose of 0.04 mg/g body weight. The dorsal aspect of the rat was prepared by shaving and bovidone iodine washing. On the dorsal aspects of the rats, caudally based random skin flaps in dimensions of 3x9 cm (surface area 2700 mm²) were elevated. In the first (control) group (n=10), the flaps were resutured to the original bed with continuous subcutaneous 4/0 polypropylene sutures (Figs. 1, 2). In the other two groups, Ischemia of the left hind limb was induced by clamping the left femoral artery and vein (Fig. 3). Following an ischemia period of 1 hour, the clamps were removed, and the return of vascular flow was confirmed by observation of the artery and vein through the microscope. After 30 minutes of reperfusion of the hind limb in the second (ARIPC) group (n=10), the flap was elevated and resutured as described in the control group. The same was done for the third (ARIPC + silicone sheet group) (n=10), but the flap was thereafter sutured back and placed onto a silicone sheet to prevent neovascularization from the wound bed (Fig. 4). The rats were housed in separate cages following the guidelines of the animal house of Faculty of Medicine, Zagazig University. Each of them had its individual source of water and standard rat food. On the seventh postoperative day all the animals were sacrificed and photographed. The flap necrosis was defined by dark colour and eschar formation. The total area of flap necrosis was measured. First, it was drawn on a transparent paper and then the areas drawn were calculated in mm². The Ethical review Committee of Faculty of Medicine, Zagazig University, Zagazig, Egypt approved the experiment.

Statistical Analysis: The mean area of flap necrosis in each group was calculated. Thereafter, the mean percentage of the necrotic area of the flap to the total flap size (2700 mm²) in each group was calculated. The difference in the mean percentage of flap necrosis (PFN) between the three groups was analyzed using Kruskal-Wallis one-way analysis of variance. The difference in the mean Percentage of flap necrosis (PFN) between two individual groups was analyzed using Mann-Whitney U-test. Probabilities of less than 0.05 were considered significant.

RESULTS

All the animals survived. No complications such as haematoma, infection or disruption of suture line developed. The necrosis became evident between the second and fourth days starting at the distal part of the flap and was well demarcated at the end of one week (Figs. 5, 6). The difference in the mean percentage of flap necrosis (PFN) between the 3 groups was highly significant ($P < 0.0001$) Table 1. The difference between the groups of ARIPC+ silicone sheet and control was not statistically significant ($P=0.073$). The difference between the groups of ARIPC and control was statistically significant ($P < 0.0001$). The same result was also found in comparing ARIPC and ARIPC+ sheet groups ($P < 0.0001$).

DISCUSSION

Ischemic preconditioning (IPC) is defined as a brief period of ischemia followed by tissue reperfusion, thereby increasing ischemic tolerance for a subsequent longer ischemic period. Several studies showed the effectiveness of classic local IPC by preclamping the flap pedicle.[15-19] There are two temporally and mechanically different types of IPC: acute preconditioning, which is induced by preclamping the flap pedicle briefly before flap ischemia,[13] and late preconditioning, induced by a preclamping procedure 24-48 h before flap ischemia.[20] However, both types of local ischemic preconditioning are difficult to be applied clinically, most likely since they can be applied only by invasive means, significantly increase operation time, or even require a second surgical procedure. The studies showed that acute IPC, enhancement of flap survival, and improvement of reperfusion in the microcirculation can be achieved not only by preclamping the flap pedicle, but also by induction of an ischemia/reperfusion event in a body area distant from the flap prior to elevation.[13,21] This new acute remote IPC (ARIPC) procedure can be applied briefly before flap elevation. The present study showed that ARIPC could augment the random pattern skin flap survival. This agrees with the results of Coban and Bulbulogu.[22] However, the mean percentage of flap necrosis in all groups in their study was generally lower than that of our study. This may be attributed to the lower mean body weight of animals in their study compared to ours. The results of our study also compares well with the results of Küntscher et al. in either skin[13] or muscle[21] flaps. Another report showed that late remote IPC using a limb tourniquet 24 h before flap ischemia attenuates ischemia/reperfusion injury in muscle flaps, whereas it was ineffective in adipocutaneous flaps[20]

Table 1. Difference in the mean birth weight and mean percentage of flap necrosis (PFN) between the 3 study groups.

Variable	Control	ARIPC	ARIPC & sheet	P
Mean Birth weight in grams	284.3 ± 0.76	287.4 ± 0.63	282.9 ± 0.82	>0.05
Mean PFN	59.15% ± 0.72	22.4% ± 0.52	53.22% ± 0.64	<0.0001

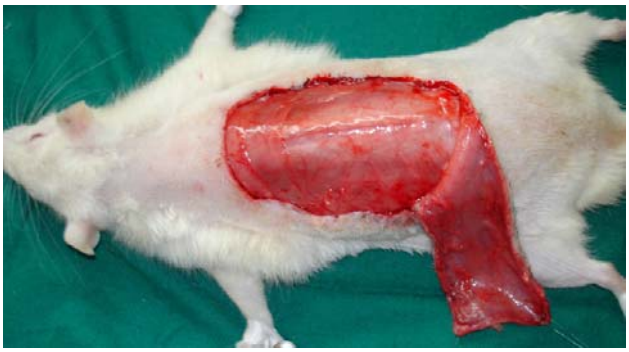

Fig 1. Caudally based skin flap on the dorsal aspect of the rate elevated.

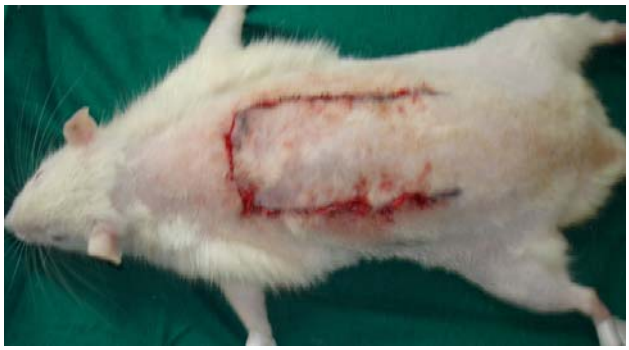

Fig 2. The skin flap resutured to its original place.

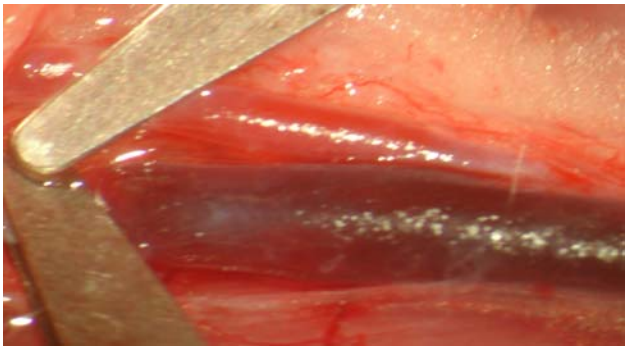

Fig 3. Femoral artery and vein of the left hind limb are clamped under the microscope.

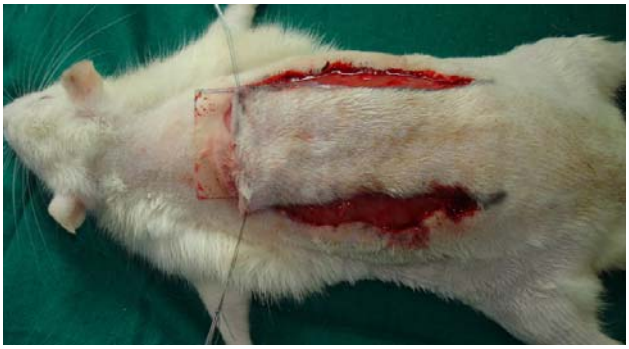

Fig 4. The silicon sheet beneath the flap before resuturing.

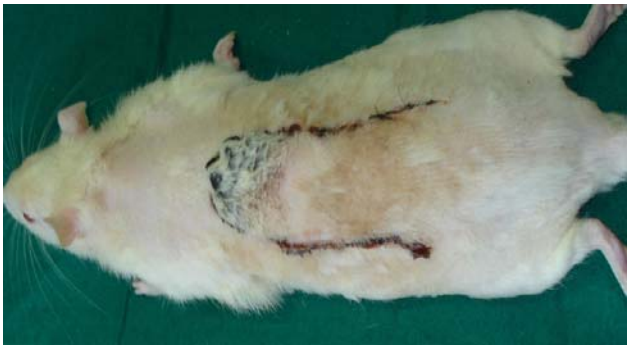

Fig 5. Usual skin necrosis pattern in the ARIPC group.

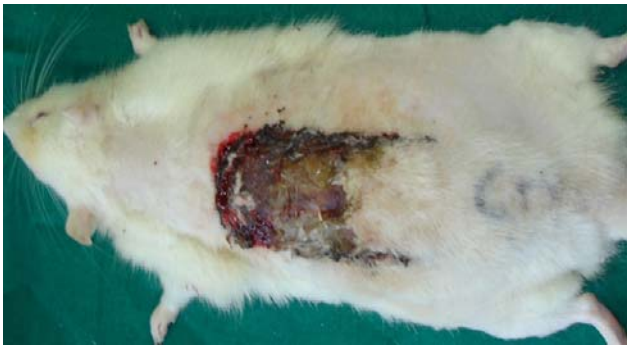

Fig 6. Usual skin necrosis pattern in control and ARIPC + silicone sheet groups.

This indicates the superiority of ARIPC over the delayed remote ischemic preconditioning. The exact mechanism of "classic" as well as remote IPC is not yet finally determined. Ischemic preconditioning (IPC) induced microcirculatory protection appears to be a systemic rather than a local phenomenon. 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 have been implicated in acute remote ischemic preconditioning.[21] The protection induced by remote ischemic preconditioning may be attributed to humoral rather than a neuronal mechanism. Ischemia followed by reperfusion in the left lower extremity of the rat induced a significant microvascular protection against subsequent ischemia in both innervated and denervated cremasters.[23] Nitric acid is believed to play an important role in ARIPC phenomenon.[24,25] In our study the enhancing effect of ARIPC on flap survival was abolished when the recipient bed was isolated. When comparing control and ARIPC + silicone sheet group, there was no statistically significant difference. In other words, isolation of the bed with silicone sheet had a negative impact on skin flap survival. The same result was reported by Coban and Bulbulogu.[22] This finding was comparable with the findings of Jones et al.[14] Although the graft effect of the bed at the flap-bed interface is controversial, factors that trigger revascularization have been found to be enhancing random skin flap survival. 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.[26,27] Adenosine treatment has been shown to augment random flap survival in rats. Adenosine is thought to be an angiogenic factor that links altered cellular metabolism caused by oxygen deprivation to compensatory angiogenesis.[28]

Vascular endothelial growth factor (VEGF) protein appears to be one of the most important angiogenic factors in vivo. It was found to be significantly increased in the skin flap with mild ischemia, but decreased in the flap with severe ischemia. Histological examination revealed increased density of the capillaries in the flaps treated with VEGF when compared to the control group.[29]

This study concluded that transient limb ischemia is a simple preconditioning stimulus that enhances random skin flap survival in rats with important potential clinical applications. The data suggest that acute remote ischemic preconditioning could be performed simultaneously with flap harvest in the clinical setting by tourniquet application to a limb, resulting in an improved flap survival without prolongation of the operation. This may decrease the rate of partial flap loss or fat necrosis, especially in high-risk groups such as smokers, those with irradiated tissues, and obese patients. Acute remote ischemic preconditioning (ARIPC) can be hypothesized to enhance the revascularization of the ischemic distal random segment of the flap. This study showed that the beneficial effect of ARIPC only works in case of a well vascularized flap recipient bed.

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