



THE EFFECT OF VASODILATORS ON SKIN FLAP SURVIVAL

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ABSTRACT**Objectives-** The aim of present study was to observe any beneficial effect of phentolamine, isoxsuprine and Sodium nitrite, on skin flap survival.**Methodology** This study was undertaken in Surgery department of L. N. Medical College, Bhopal. Twenty four rats of both sexes were taken and study was performed in three Groups. Flaps treated with Phentolamine, Isoxsuprine and sodium nitrate. After 8 days dressings were removed, the length of surviving flap was measured. The flaps were sent for histological study for the evidence of dilatation of blood vessels in the skin flaps.**Results** In the flaps treated with Phentolamine, there was slight evidence of neovascularization. While in the flaps treated with Isoxsuprine, there was marked evidence of neovascularization. In the flaps treated with Sodium nitrite neovascularization was of moderate degree. Phentolamine produced +29.26% more flap survival over controls, whereas isoxsuprine produced +26.74% and sodium nitrite +17.46% better flap survival**Conclusion.** The drugs are cheaper, easily available and least toxic. More sophisticated methods, delicate and careful handling of the tissues and intelligent use of these vasodilators may produce better flap survival, and reduce anxiety and worries of the surgeons concerned**KEYWORDS :** Phentolamine, Neovascularization, Isoxsuprine, Sodium nitrite**INTRODUCTION**

It is now firmly established that skin is the best possible dressing for a raw surface. Where there is actual loss of skin resulting from trauma or from operations for the removal of malignant growths, immediate skin cover is often required.

The present work is concerned with third method of plastic surgery i.e. covering the defects by pedicle flaps. The method of covering raw areas and defects is applicable when the donor area and the recipient area can be brought into close proximity with each other. By its use, skin can be transferred from the trunk to the upper limb, or from the one lower limb to the other.

Transfer of a flap is a basic procedure in reconstructive surgery and survival of the flap is critical for the success of the procedure. Among surgeons concerned with these problems, it is a matter of common knowledge that mechanical insult to the flap such as tension and kinking are detrimental and not infrequently lethal to the flap. Avoidance of these types of obvious and direct trauma is imperative. Unexplained losses of flaps occur because our knowledge of the factors which bear on circulation in flaps is limited. (Fujno 1967)[1]

Based on the assumption that flap necrosis is secondary to an inadequate blood supply, investigators have sought methods of improving flap perfusion. However measures which supposedly increase circulation have not been successful in preventing flap necrosis. When the pedicle flap is designed carefully and correctly the circulation will be satisfactory from the moment it is raised, occasionally the circulation in distal end is compromised- the so called - "Frightened flap"- but the colour gradually improves within a few hours of operations. If the circulation is less satisfactory, some portion of the distal end of the flap will become necrotic although the viability may remain in doubt for two or three days.

In the present study, by the use of some vasodilator drugs, it has been tried to prevent or at least minimize the flap necrosis. The assumptions that catecholamines are at least partially responsible for skin flap necrosis, raised the questions, whether the use of - adrenergic blocking agents such as Phentolamine (Regitine), β -receptor stimulating agents, such as Isoxsuprine or agents which directly act on vascular smooth muscle such as sodium nitrate, will overcome this harmful effect of catecholamines.

Considering our limited resources we have chosen albino rats for the present study, since we consider the human subject a costly machine for which there are no spares. May be, if we are successful in our errand, we shall be able to provide sufficient number of spares to the ailing masses in our surgical workshop, rendering them the status of useful and functional units in the ocean of humanity.

METHODOLOGY

The study was performed in three Groups:

Group 'A' Consisted of eight albino rats. In this group, on the left side of vertebral column over back of the human, control flap was designed. After eighty days, on the right side over back, Phentolamine (Regitine) was injected, 2.5mg/kg. in one ml. volume four hours prior to the experiment, at the site where base of the flap was to be designed.

Group 'B' This also consisted of eight albino rats. On the left of vertebral column control flap was designed. After 8 days, Isoxsuprine (Duvadilan) 2.5mg/Kg in the volume of 1 ml was injected at the base of the flap 4 hours prior to the experiment on the right side of vertebral column.

Group 'C' Consisted of eight rats. Same as group 'A' and group 'B' control flap was designed on the left side of vertebral column and after 8 days Sodium nitrite, freshly prepared, 2.5mg/kg in 1ml volume was injected on the basis of the flap on right side, 4 hours prior to the experiment.

CONTROL FLAPS On the day of operation the weight of the rats was recorded. The back of rats was shaved from neck to tail extending upto the flanks with the help of scissors avoiding injury to the skin. Rats were anaesthetized by ether, in a cotton wool ball, kept over the nose.

Design of flaps On the left side of vertebral column. The width of flap was 1 cm and was kept constant in all experiment. An incision was made parallel to the vertebral. Another incision was made 1 cm lateral to the first one and parallel to it. Both the incisions were joined by a transverse incisions at the distal end of flaps. The flaps were handled with great care and no traumatizing instrument was used. The flap was designed as long as possible, but usually length

was kept from 6 to 8 cms. The flap was dissected free in the cleavage of connective tissue between the subcutaneous muscle plate – the penniculus and the muscles of the back. There was no marked bleeding during dissection. The flap was then returned back to its bed, and sutured there with the help of eight fine silk sutures (2-0), four on each side, on a traumatic needle.

Sterilized dressings were applied. On 8th day dressings were removed, the length of surviving flap was measured. The same rat was again used for design of flap treated with one of the vasodilator drugs used: on the right side of vertebral column.

FLAPS TREATED VASODILATOR DRUGS:

After 8 days of control flap experiment, the same rats were used. The back was again shaved. Weight was recorded. The drug used, was injected on the right side of vertebral column, on the base of the flap, in 1 ml volume. 2.5 mg/kg, subcutaneously and intradermally, 4 hours prior to the experiment.

After 4 hours, rats were anaesthetized, secured to the operating board, and a flap of same size as in control flap, was dissected on the right side with great care, and sutured back as the control flap was. After 8 days dressings were removed, the length of surviving flap was measured. Some of the flaps from each group, along with their control flaps were sent for histological study for the evidence of dilatation of blood vessels in the skin flaps treated with vasodilator drugs as compared to their control flaps.

Rats were again anaesthetized and flaps from both the sides were removed carefully. The tissues were kept in 10% Formal saline and then were sent to department of Pathology, L N Medical College for histology. The tissue was impregnated in paraffin, blocks were made. Sections were stained with haematoxyline and eosin.

OBSERVATION TABLES

TABLE -1: SURVIVAL IN CONTROL FLAPS (GROUP 'A')

S. No.	Initial length of Flap	Length of surviving Flap (after 8days)	%
1	8 cms	5.6 cms	70%
2	8 cms	5.4 cms	67.5%
3	8 cms	5.65 cms	70.62%
4	8 cms	5.5 cms	68.75%
5	8 cms	5.54 cms	69.25%
6	8 cms	5.6 cms	70%
7	8 cms	5.52 cms	69%
8	8 cms	5.75 cms	71.87%

Mean 5.57 cms. Mean 69.62%

TABLE 2: COMPARISON OF FLAP SURVIVAL IN CONTROLS AND PHENTOLAMINE TREATED.

S. No.	Flap	Mean Survival	Mean Percentage
1	Control	5.57 cms	69.62%
2	Phentolamine treated	7.20 cms	90.00%

Enhancement of flap survival in phentolamine Treated flap was +29.26%

TABLE - 3: ENHANCEMENT OF SURVIVAL IN FLAPS TREATED WITH ISOXSUPRINE AS COMPARED TO THEIR CONTROLS.

S. No.	Percentage of Survival in control flaps.	Percentage of survival in treated flap.	Enhancement of flap survival by Isoxsuprine
1	75.00%	90.00%	+20%
2	65.00%	91.66%	+41.01%
3	70.00%	83.75%	+19.64%
4	66.25%	81.25%	+22.64%
5	71.25%	87.50%	+22.80%
6	66.25%	82.50%	+24.52%

7	65.00%	86.25%	+39.69%
8	68.75%	85.00%	+23.63%

Mean = +26.74% The mean surviving length in control flaps was 5.12 cms, whereas it was 6.42 cms in the flaps treated with 2.5 mg/kg of Isoxsuprine. The effect of Isoxsuprine was +26.74% more survival in the flaps as compared to their controls.

TABLE- 4: COMPARISON OF FLAP SURVIVAL IN CONTROLS AND IN ISOXSUPRINE TREATED.

S. No.	Flap	Mean Survival	Mean Percentage
1	Control	5.12 cms	68.43%
2	Isoxsuprine Treated	6.42 cms	85.98%

The average enhancement of flap survival by Isoxsuprine was +26.74%.

TABLE - 5: ENHANCEMENT OF SURVIVAL IN FLAPS TREATED WITH SODIUM NITRATE AS COMPARED TO THEIR CONTROLS.

S. No.	Percentage of survival in control flaps	Percentage of survival in treated flaps	Enhancement of flap survival sodium nitrate
1	68.75%	Died	
2	70.62%	78.75%	+11.5%
3	72.50%	83.75%	+15.5%
4	67.50%	81.25%	+20.3%
5	65.00%	76.25%	+17.69%
6	68.75%	77.50%	+12.72%
7	62.50%	75.00%	+20.00%
8	66.25%	82.50%	+24.56%

Mean Enhancement of flap survival = +17.46%

TABLE-6: COMPARISON OF FLAP SURVIVAL IN CONTROLS AND SODIUM NITRITE TREATED.

S. No.	Flap	Mean Survival	Mean Percentage
1	Control flaps	5.41 cms	67.73%
2	Sodium nitrite Treated	6.34 cms	79.28%

Enhancement of flap survival in Sodium nitrite flaps was +17.46%. The mean length of surviving flap was 5.41 cms in controls flaps, whereas it was 6.34 cms in the flaps treated with sodium nitrite. The effect of Sodium nitrite was +17.46% more survival of flaps as compared to the controls.

TABLE - 7: COMPARISON OF FLAP SURVIVAL IN ALL THE THREE GROUP.

S. No.	Group	Mean Surviving length	Mean Percentage of flap surviving	Enhancement of flap survival
1	'A' Phentolamine	7.20 cms	90%	+29.26%
2	'B' Isoxsuprine	6.42 cms	85.98%	+26.74%
3	'C' Sodium nitrite	6.34 cms	79.28%	+17.46%

TABLE - 8: COMPARISON OF CONTROLS AND TREATED FLAPS SURVIVAL IN ALL THREE GROUPS.

S. No.	Group	Control flap survival	Treated flap survival	Enhancement of flap survival
1	'A' Phentolamine	69.62%	90%	+29.26%
2	'B' Isoxsuprine	68.43%	85.98%	+26.74%
3	'C' Sodium nitrite	67.73%	79.28%	+17.46%

RESULTS

In the flaps treated with Phentolamine, there was slight evidence of neovascularization. While in the flaps treated with Isoxsuprine, there was marked evidence of neovascularization. In the flaps treated with Sodium nitrite neovascularization was of moderate degree.

Phentolamine produced +29.26% more flap survival over controls, whereas isoxsuprine produced +26.74% and sodium nitrite +17.46% better flap survival. Group –A: flap survival was 90% and it was +29.26% more, over the control flaps. Group- B: flap survival was 85.98% and it was +26.74% more, over control flaps. Group- C: Flap survival was 79.28% and was +17.46% more over control flaps.

STATISTICAL ANALYSIS-

The two-tailed P value was taken as the criteria to calculate significance. Comparison between various pre-test and post-test values P value and statistical significance: The two-tailed P value is less than 0.0001 was considered significant. By conventional criteria, this difference is considered to be extremely statistically significant.

DISCUSSION

Several methods have been described which protect the distal end of a pedicle flap. The delay procedure accomplishes this by conditioning the tissues to withstand a period of hypoxia during the time that the circulation is becoming reestablished. Mc Farlane et al 1965.[2]

Significance of the reactive oxygen species in distal flap necrosis and the efficacy of superoxide dismutase (SOD) in salvaging necrosis have become indicated in recent studies. To enhance the therapeutic effect of SOD, topical application was designed. Water-soluble ointment containing 1% SOD was prepared, and three experiments were made to study the effect of topical SOD in improving flap survival. Simple application of SOD ointment to the upper surface of the flap had no effect, whereas its application to the under surface or occlusive dressing was effective. These effects were corroborated by assay of tissue SOD activity.[3]

Bunke (1965) and Krizek (1965) added sophistication to the transfer of a pedicle flap by anastomosing the major artery and vein of the flap at a distant site while others reported that dimethyl sulphoxide, applied topically, reduced the amount of necrosis, in a pedicle flap. Many reports like Wolfort SF, Angel MF et al state that low molecular weight dextran improves blood flow at the capillary level by preventing or reversing sludging of red blood cells [4]

Black CE, Huang N et al study tested the hypothesis that acute exposure of human skin vasculature to nicotine may have deleterious effects on endothelial function. Vasoconstriction and vasorelaxation in isolated perfused human skin flaps derived from dermolipectomy specimens were assessed by studying changes in skin and skin perfusion was assessed by a dermo-fluorometry technique. It was observed that nicotine amplified the norepinephrine induced concentration-dependent increase in skin vasoconstriction compared with the control. It was also observed that ACh and nitroglycerin (NTG) elicited a concentration-dependent vasorelaxation in skin flaps precontracted with NE. These findings may provide further insight into the pathogenesis of skin vasospasm in skin flap surgery and skin ischemic disease associated with cigarette smoking or use of smokeless tobacco.[5]

Similar studies were done by Finseth F, Adelberg MG et al They also worked on prevention of skin flap necrosis by a course of treatment with vasodilator drugs. Their results were similar to our studies. Rohrich RJ, Cherry GW et al did enhancement of skin-flap survival using nitroglycerin ointment. [6,7]

Padubidri A, Browne Jr evaluated the efficacy of VEGF in augmentation of blood supply to skin flaps. In group A (N = 8), 5 [µ]g of VEGF was injected into the epigastric artery after flap elevation. In the control, group B (N = 8), the artery was injected with saline. On the seventh day, blood flow in the flaps was measured with a percutaneous laser Doppler probe at specific locations. Histological studies of the flaps were done. Results showed that the mean percentage surviving flap area was 71.9% in group A and 53.7% in the control group, which is statistically significant (p < 0.001). Histological examination revealed increased density of the

capillaries in the flaps treated with VEGF when compared to the control group. This study shows the increase in skin survival is due to angiogenesis induced by the VEGF.[8]

Emery FM, Kodey et al did their study on Nifedipine, a calcium-channel blocker. It is a peripheral vasodilator and has been shown to increase blood flow to skin. The hypothesis that nifedipine would thereby improve skin-flap viability was tested by comparing the extent of necrosis of long pedicle flaps in control and nifedipine-treated rats. Thirty rats were randomized to receive either 2.5 mg/kg nifedipine. Serum nifedipine levels were determined by gas chromatography. The extent of distal flap necrosis was expressed as a percentage of the total flap area, and differences were studied by one-way analysis of variance.[9,10]

Gardiner SM, March JE in similar studies saw the regional haemodynamic effects of rat or human urotensin II. Rat and human U-II had similar effects. At doses of 300 and 3000 pmol kg⁻¹, there were dose-dependent tachycardias, and mesenteric and hindquarters hyperaemic vasodilations. Thus, in conscious rats, the predominant cardiovascular action of rat and human U-II is vasodilation. This is in contrast to recent findings with human U-II in non-human primates, but is consistent with effects on human isolated resistance vessels.[11]

Gill PS, Hunt JP, Guerra AB in their study examined 758 deep inferior epigastric perforator flaps for breast reconstruction, with respect to risk factors and associated complications. Risk factors that demonstrated significant association with any breast or abdominal complication included smoking (p = 0.0000), postreconstruction radiotherapy (p = 0.0000), and hypertension (p = 0.0370). Ninety-eight flaps (12.9 percent) developed fat necrosis. Associated risk factors were smoking (p = 0.0226) and postreconstruction radiotherapy (p = 0.0000). They concluded that with experience in microsurgical techniques and perforator selection, the deep inferior epigastric perforator flap offers distinct advantages to patients, in terms of decreased donor-site morbidity and shorter recovery periods[12]

Lineaweaver WC, Lei MP, Mustain W et al in their research study concluded that surgical delay significantly increases skin paddle survival in the delayed TRAM flaps compared with undelayed flaps. TGF-β and PDGF expressions were not changed by surgical delay, but basic fibroblast growth factor (bFGF) and VEGF expressions increased significantly. In the rat TRAM model, surgical delay resulted in increased VEGF expression and increased skin paddle survival. These results correlate with previous studies showing the preoperative injection of VEGF increases skin paddle survival. VEGF may be an important element in the delay phenomenon and may be an agent for pharmacological delay.[13]

McDonald WS, Lo Jr TP, Thurmond M et al in similar study concluded that surgical delay of skin flaps before transfer is known to improve flap viability. This study attempts to elucidate the mechanism of vasodilation by exploring the effects of nitric oxide on the microcirculation of delayed skin flaps. Vessel flow was measured using an optical Doppler velocimeter. Next, each animal received an intraperitoneal dose of nitro-amino-methyl-L-arginine (L-NAME), a nitric oxide synthase inhibitor. Vessel diameter increased to 44.92 µm in the control (0 mg/kg L-NAME) animals. Flow increased to 7.66 nl/sec in the control animals. Vessel dilation and flow did not change significantly in the nonoperative vessels. As the dose of L-NAME increased in the treated animals, there was a significant decrease in vasodilation and flow (p = 0.015 and p = 0.03, respectively). The results demonstrate that nitric oxide is an important element of vasodilation and contributor to the phenomenon of skin flap delay.[14]

When studying about recent trends we consider study done by Hart K, Baur D, Hodam et al who studied short-and long-term effects of sildenafil on skin flap survival in rats and concluded that PDE 5 inhibitors may play a more important role in early postoperative

skin flap viability rather than at later time points and may be beneficial for skin flap viability as shown in the rat model. PDE 5 inhibitors may reduce the extent of necrosis after reconstructive surgeries.[15]

CONCLUSION:-

There vasodilator drugs were used to increase the flap survival in rats, since flap necrosis is challenging problem to the surgeons engaged in this type of work. The aim of present study was to observe any beneficial effect of phentolamine, isoxsuprine and Sodium nitrite, on skin flap survival. Rats were chosen for the work as they are easily available, easy to handle and their cutaneous vasculature simulates that of human in many respects. Phentolamine produced +29.26% more flap survival over controls, whereas isoxsuprine produced +26.74% and sodium nitrite +17.46% better flap survival. The drugs are cheaper, easily available and least toxic.

More sophisticated methods, delicate and careful handling of the tissues and intelligent use of these vasodilators may produce better flap survival in human, and reduce anxiety and worries of the surgeons concerned

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