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Comparing the Effect of Hirudoid on Random Skin Flap Survival in Rats Based on Different Application Times

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Abstract

Objectives: The objective of the present study was to compare the use of Hirudoid immediately after surgery with its use 6 hours post-operatively when probable thrombosis was formed but tissue damage was still reversible.
Methods: After preparing 3 × 11 cm dorsal flap in all rats, one group of 8 rats received Hirudoid topically immediately after surgery during 9 post-operative days. Another group received it 6 h after surgery during 9 post-operative days. The control group received Vaseline immediately after surgery on a daily basis during 9 post-operative days.
Results: The mean area of flap survival in the control group was 8.75 ± 3.32 cm^2 (mean ± SD) and it was 12.38±1.93 cm^2 and 14.36 ± 3.51 cm^2 in Hirudoid immediately after surgery and 6 hours after surgery groups, respectively.
Conclusions: It was found that Hirudoid can be effective in improving flap viability; although its effect was not statistically significant when used immediately after surgery ($P > 0.05$), it significantly increased flap survival when used 6 h after surgery ($P = 0.003$).

Keywords: Skin Random Flap, Ischemia, Flap Necrosis, Hirudoid

1. Background

Random skin flap is one of the most common operations in plastic and reconstructive surgery (1). Traumas and oncologic ablations often cause a significant loss of soft tissue accompanied by aesthetic and functional deficits. Regional, pedicle, and free flaps are main surgical approaches for reconstructing these defects (2). Flaps are more appropriate than skin graft because of their color, thickness, consistency, and more similarity to the main tissue (1). While using random regional or pedicle flaps, length to width ratio is important and an inappropriate design and surgical technique result in ischemic necrosis, wound dehiscence, infection, delayed wound healing, longer hospitalization period, more surgeries, several visits, more morbidity, and higher costs (1). Controlling some factors like smoking and the nutritional status of the patient, avoiding excessive electro cautery, ensuring adequate homeostasis, and preventing tissue injuries such as damage to axial vessels, torsion of the flap pedicle, excessive tension on closure, and strangulation of pedicle during surgery are very important in preventing flap necrosis (2). Width to length ratio is a limiting factor in this type of reconstruction (3). Delayed surgeries can increase flap survival with documented results but more than one operation is needed (3). Various pharmacological agents have been investigated in preventing flap necrosis which include sympathlytics, vasodilators, calcium channel blockers, hemorrheologic agents, prostaglandin inhibitors, anticoagulant agents, glucocorticoids, and free radical scavengers, most of which act by inducing vasodilation and increasing perfusion (4). These medications are more effective in systemic application with high doses and, therefore, have more systemic side effects (5, 6). Various mechanisms including vasospasm, endothelial cell damage, thrombus formation in microvasculature, and ischemia-induced tissue damage take part in the pathogenesis of flap necrosis. Failure in flap surgery still occurs in 5% - 10% of cases because of vasospasm and thrombosis. Vasospasm has an important role in the pathogenesis of thrombosis formation and causes ischemic necrosis in flap which can occur during the surgery and after 72 hours (4). Primary ischemia resulted by flap elevation is an important factor for producing flap necrosis, but most flap necrosis develop due

to other reasons (2). The activation of the coagulation system by trauma to microvasculature causes flap necrosis (6). Another leading cause is the increase in neutrophil adhesiveness. Tissue injuries can clog microvasculature by neutrophils; then, injury cascade initiates by releasing free radicals, enzymes and cytokines physically injure the endothelium, obstruct capillaries, and block oxygen supply to the tissue (3). Perfusion pressure and vascularity in the flap pedicle determine flap viability. Previous studies have recommended that drugs which are beneficial in flap survival are better to be used in the first 8 hours before the cellular damage is irreversible (7). Finding a safe, easy, and cost-effective way to prevent flap necrosis is necessary (1). Hirudoid is a topical cream; the active ingredient of it is mucopolysaccharide polysulphate (MPS), a semi-synthetic molecule that is produced by the sulphation of a glycosaminoglycan mixture obtained from mammalian cartilage. The substance composed of aminosugars is chemically linked to repeating units which form a linear un-branched polymer. Because of its chemical relationship with heparin, MPS has been frequently described as a heparinoid. Hirudoid is used as an anticoagulant, fibrinolytic, and anti-inflammatory agent (3). These three characteristics which are simultaneously available in a topical cream and are easily operable, encouraged the present authors to investigate the effect of this drug on flap viability in rats. There was only one study in the literature which showed that Hirudiod increased flap viability (3).

2. Objectives

The present study was designed to compare the use of Hirudoid immediately after surgery with its use 6 hours post-operatively when probable thrombosis was formed but tissue damage was still reversible.

3. Methods

This experimental study was performed on 24 male Spague-Dawley rats of the same age which weighed between 300 and 350 gr without any diseases. All the animals were treated in compliance with the recommendations of the 7^{th} edition of "guide for the care and use of laboratory animals" published by NRC (1997) and the 2^{nd} edition of ARENA/OLAW "institutional animal care and use committee guide book" (2002). The animals were housed individually after surgery to prevent skin flap cannibalism. Water and standard laboratory food for rats were provided ad libitum. To the environmental stress (8), all the rats were transported to the laboratory at the same time and were kept for ten days. Then, they were randomly assigned to three groups and anesthetized with intramuscular injection of 90 mg/kg Ketamine (Ketamine 10%, Alfasan lab, Woerden, Netherland) and 9 mg/kg Xylazin (Xylazin 2%, Alfasan lab, Woerden, Netherland). The skin of dorsal trunks were shaved with electric clippers and then prepared with betadine (9). Before the surgery, 60 mg/kg intramuscular injection of cephazoline was used as prophylactic antibiotic (4). Depth of anesthesia was confirmed by the pinch flexion/withdrawal test (10). Caudally based 11×3 cm sized dorsal flaps (as described by McFarlane et al.) (11) were raised under sterile conditions. Palpable hip joints were marked as the basis of the flaps which were dissected and detached by their Panniculus Carnosus (10). All perforating and axial vessels were cut and sterile drapes (Incifilm, Pharmaplast, Alexandria, and Egypt) were placed in the wound bed to prevent flap survival by graft effect. Then, the flaps were re-placed in the original position and repaired by separate sutures.

In the first group, first, immediately after the surgery and then for 9 post-operative days on a daily basis, 2 g (1 cc) Hirudoid cream (Hirudoid®, Sankyopharmamünchen, Germany) was administered topically on the flap surface. In the second group, the drug was used 6 h after the surgery and daily for the next 9 post-operative days. The amount of the drug in both groups was 2 g (1 cc) per day. In contrast, in the control group, Vaseline was applied daily on the flaps. Vaseline was chosen as the control ointment because it did not have any known pharmacological properties (6). At the end of the 9th day, the animals were re-anesthetized and, after digital photography (by a Nikon D 300 digital camera, 60 mm Nikon macro lens, 1: 10 enlargement and 80 cm distance using ruler in the field) (Figure 1), they were killed. The alive part of each one was measured by Image J v.1.40 g (NIH, USA) after calibration.

3.1. Statistical Analysis

The results were expressed as mean $\pm$ SD and the data distribution in each group was normal according to Kolmogorov-Smirnov ($P > 0.05$) test. To compare the groups, ANOVA (analysis of variance) was performed.

4. Results

All the rats survived until the end of the study and no infection was encountered. The mean area of the flaps' alive parts was 8.75 ± 3.32 cm^2 (Mean $\pm$ SD) in the control group, 12.38 ± 1.93 cm^2 in Hirudoid immediately after surgery and 14.36 ± 3.51 cm^2 in Hirudoid 6 h after surgery group (Figure 2, flap survival area).

The mean flap survival was $26.52 \pm 10.08\%$ (Mean $\pm$ SD) in the control group, $37.52 \pm 5.86\%$ in Hirudoid immedi-

Figure 1. On Post-Operative Day 9; the Group Treated With Hirudoid Immediately After Surgery, the Group Treated With Hirudoid 6 Hours After Surgery and the Control Group

ately after surgery and 44.14 ± 10.63% in Hirudoid 6 hours after surgery.

These results showed that, although the administration of Hirudoid cream immediately after surgery increased flap survival, there was no statistically significant difference between the rats in this group and those in the control group (P = 0.062). Using Hirudoid 6 hours after surgery increased flap survival in comparison with the control group (P = 0.003). Using Hirudoid 6 hours after surgery showed better results than applying it immediately after surgery, but it was not statistically significant (P = 0.4).

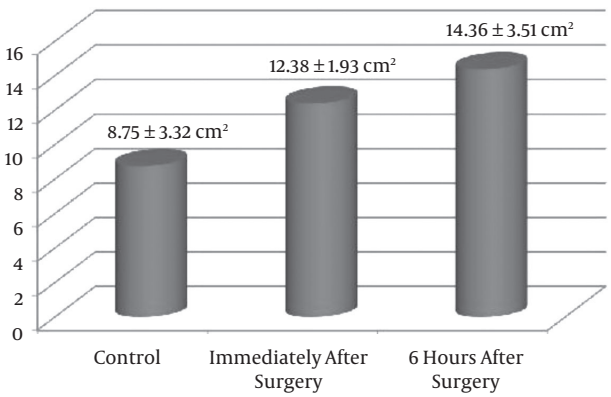

Figure 2. Flap Survival Area (Mean + SD)

5. Discussion

Local random flaps are the best surgical choices for reconstructing defects after surgery or trauma, particularly in head and neck reconstruction, in which a tissue with similar color and consistency is required more. In flap design, length to width ratio is important. Appropriate proportion depends on the vascularity and sub dermal plexus of each area, about 3 - 4 to 1 length to width (10). However, in every condition, flap ischemia and necrosis may occur (8). Many drugs have been studied in preventing flap ischemia and necrosis. These drugs are classified in 8 groups including sympatholytics, vasodilators, calcium channel blockers, hemorrhologic agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free radical scavengers (10). For investigation, rats are mostly used because of their easy operability and cost-effectiveness (8). However, in these studies, flaps have a wide range of viability in control and treatment groups. Differences in race, sex, and feeding order of rats, design of flap, time of study, amount of drug, and infection after surgery can explain this variety (8) and should be considered in concluding the results.

Mucopolysaccharide polysulfate or Hirudoid is a topical cream which represents anticoagulant, fibrinolitic, and anti-inflammatory effects simultaneously (3); therefore, seemingly, it is able to prevent flap necrosis and improve flap survival. Systemic administration of this drug, similar to intravenous use of Heparin, has antithrombotic effects and increases bleeding risk but has less anti-inflammatory effects (12, 13). Previous studies have shown that Hirudoid decreases thrombosis formation both in systemic and local administration (13). This drug penetrates into the skin of humans and animals and also enters systemic circulation and affects the coagulation system (3). In the present work, use of Hirudoid immediately after surgery increased flap viability, but it was not statistically significant, which differed from the results of Livaoglu et al. (3). They had 3 × 10 cm flaps on Wistar rats without using sterile drape; in contrast, there were elevated 3 × 11 cm flaps on Sprague-Dawley rats and sterile drapes were used under the flaps to prevent graft effect, which can improve flap survival. The rats of the present study were kept in the animal laboratory 10 days before the surgery in order to decrease protective effects of transporting stress (8). Also, 2 gr Hirudoid versus 0.5 gr was used for their study. The differences in methods can explain various results. In Livaoglu et al.'s study, histopathological evaluations showed lower inflammation and congestion in flaps in the treatment group which could be the consequence of better micro vascular perfusion by diminishing intravascular thrombosis. As expected, using Hirudoid immediately after surgery improved flap survival although it was not statistically sig-

nificant. Livaoglu et al.'s investigation was the only study in the literature which investigated the effect of Hirudoid on random flaps. All other previous studies are about phlebitis and hematoma.

Using Hirudoid 6 hours after surgery improved flap viability significantly in comparison with the control group, which was not the case with using Hirudoid immediately after surgery. It was used 6 h later to have its anticoagulation and fibrinolytic effect before cellular damage was irreversible; thus, better results were obtained in this kind of administration.

Thrombosis formation took place after flap surgery anyway; so, using a good absorbable topical thrombolytic and anticoagulant can reduce ischemia caused by microhematomas and thromboses without systemic side effects and lead to no increased bleeding. Additionally, the anti-inflammatory effect of this drug probably had a role in improving flap viability by affecting inflammatory mediators in the region. Further studies will determine this effect and its mechanisms.

5.1. Conclusion

Based on this study and Livaoglu et al.'s results, Hirudoid improved flap viability. More studies are required to clarify these equivocations. This paper recommended using Hirudoid in a well-designed clinical trial in three different periods of before, during, and after surgery in order to help understand the effects and the mechanisms of Hirudoid for increasing the flap survival area.

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Footnotes

Authors' Contribution: Study concept and design: Babak Nikoumaram and Mohammad Javad Fatemi; acquisition of data: Shahrzad Taghavi and Shirin Araghi; analysis and interpretation of data: Zeinab Nematzadeh; drafting of the manuscript: Bahareh Salehi; critical revision of the manuscript for important intellectual content: Mohammad Javad Fatemi; statistical analysis: Mohsen Saberi; study supervision: Mohammad Javad Fatemi.

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