의학석사 학위논문

The Effect of Substance P on Skin Flap Survival in Rats

쥐에서 P 물질이 피부 피판의 생존에 미치는 영향

2013 년 2 월

서울대학교 대학원

의학과 성형외과학 전공

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A thesis of the Master of Science in Medicine

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February, 2013

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ABSTRACT

Introduction: Skin flap necrosis is a challenging problem in reconstructive surgery. Pharmacologic administration that increases skin flap survaival has been investigated extensively. It has been reported that substance P, a member of tachykinin family neuropetides, stimulates vessel growth in a variety of angiogenesis assays. The purpose of this study was to evaluate the effects of local administration of substance P on skin flap survival in rat model.

Materials and Methods: Twenty-four male Sprague-Dawley rats were randomly divided into two groups of twelve. Rats were anesthetized, and a skin flap, measuring 10 × 3 cm was designed on the rat dorsum. After local injections of 1ml substance P (100nmol) or normal saline (control) at the distal half, the skin flaps were elevated. Then the flaps were immediately sutured back in situ. 7 days after the flap elevation, survival rate of the flap was calculated and a histological examination was performed.

Results: The mean survival rate of flaps in substance P administration group (80.4±7.0%) was significantly higher than

that in control group (61.9±6.9%)($p<0.001$). Histological observation of the flaps showed increase of vessel number in the group treated with substance P when compared with the control group.

Conclusion: The results demonstrate that the use of local administration of substance P increases skin flap survival in rats by enhanced vessel number.

Keywords: Substance P, Skin flap survival, Angiogenesis.

Student number: 2011-23017

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INTRODUCTION

Skin flaps have been used extensively as a reconstructive option in the field of plastic surgery. However, in all skin flaps, distal flap necrosis resulting in failed reconstruction remains a serious complication. This applies particularly for the distal part of the flap that covers the initial defect. Many factors are related to the flap necrosis, involving some local and systemic causes, but in most cases, the main reason is arterial insufficiency, venous congestion or both.

Many approaches have been attempted to reduce distal part flap necrosis. Among them, flap delay is probably the most effective method but requires at least one additional surgical intervention with its inherent drawbacks. Another approach is the perioperative use of pharmacologic agents to enhance flap perfusion and survival. Studies have investigated the effect of systemic, local, and combined pharmaceutical applications on flap survival. It has been shown that an important factor of flap survival is the effect of angiogenesis.

Substance P is a member of tachykinin family neuropeptides which are small molecules secreted from the peripheral

terminals of sensory nerve fibers and act as neurotransmitter or hormone[1]. It mediates pain perception[2], regulates wound healing[3] and has a potential to induce angiogenesis[4-8].

It has been demonstrated that substance P, via direct or indirect effects on endothelial cells, induces vasodilation and plasma extravasation in vivo[9]. Migration and proliferation of endothelial cells[10], as well as proliferation of smooth muscle cells is induced by substance P in vitro[11 12]. And also substance P-enhanced endothelial cell proliferation and in vivo angiogenesis are mimicked by selective NK1 receptor agonist and inhibited by antagonists of neurokinin receptors[13].

Although it has been demonstrated that substance P has multifunctional nature of both angiogenic and chemotactic activities, there is no experimental report about the effect of local administration of substance P on skin flap survival.

In this regard, the author hypothesis that local administration of substance P might be beneficial in reducing necrosis in ischemic skin flaps and enhancing the survival rate of skin flap in rat model.

MATERIALS AND METHODS

Animals and Treatment

Twenty four male Sprague-Dawley rats weighting around 300g were used in this experimental study. The rats received humane care in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council) and were managed in a regulated airflow room, where temperature, humidity, and light were controlled. The rats were randomly divided into two groups of twelve each. All rats were anesthetized with intramuscular injection of ketamine (75mg/kg) and xylazine (3mg/kg), and then the dorsal trunk was shaved with electric clippers. From the dorsum of the prepared rats, a cranially based 10 × 3cm flap was designed with its base on the lower margin of the scapula. In the treatment group, 1ml of substance P (Sigma-Aldrich Co., St. Louis, MO) solution (100nmol) was subcutaneously injected into ten marked sites (0.1ml/site) at the distal half of the flap (Figure 1). In the control group, 1ml of normal saline was injected by the same method. After injection of the substance P or normal saline, the flap including panniculus carnosus muscle was elevated. To

prevent the angiogenesis from the bed, a silicone sheet (Bioplexus corporation®, Ventura, CA) was placed on the flap bed. After hemostasis, the flap was sutured back with a 4-0 nylon suture to its original location. To avoid the possibility that the rats would bite the flaps after they recovered from anesthesia, each rat was placed in separate cage.

Evaluation of skin flap survival rate

The flap survival rate was evaluated on postoperative day 7. The rats were sacrificed and a writable transparent paper was placed over the dorsum of each rat, accounting for the curvature of the dorsal surface, and the flap was traced taking care to draw the line of demarcation between the viable proximal flap tissue and the necrotic distal portion. Flap tracing analysis was performed digitally after each flap tracing was scanned into a computer as an image file. The images were then analyzed using ImageJ software (Wayne Rasband, NIH) to determine the percentage of survival rate of each flap. A straight line was digitally drawn connecting the tracings representing the cranial ends of the lateral incisions. Results were expressed as percentages of surviving area in relation to

the total flap surface area.

Histological evaluation

7 days after the flap elevation, skin specimens were taken from the surviving district near the demarcation of survival and necrotic areas, 1×1cm in size. And then specimens were fixed in 10% formaldehyde solution for 24h, embedded in paraffin for hematoxylin and eosin (H&E) staining to detect capillaries in each group. Angiogenesis was assessed by averaging the number of mature vessels containing erythrocytes in subcutaneous tissue in 5 randomly chosen fields of microphotograph (200 × magnification).

Statistical evaluation

Statistical analysis was performed using SPSS version 19 statistical software. Each measurement is shown as mean±standard deviation. All pairwise differences between the measurements of two groups were examined by a Mann-Whitney test. A p-value of <0.05 was considered statistically significant.

Results

Survival rate of skin flap in rats

According to the examination of the flap survival on postoperative day 7, all flaps showed clear demarcation between necrotic and survival areas (Figure 2). And distal areas of skin flap that were hard or covered with crust were judged to be necrotic. The mean percentage of the survival area in the substance P treatment group was 80.4±7.0%, and the mean of the control group was 61.9±6.9% (Figure 3). The results between the group injected with substance P and the control group were statistically significant ($p < 0.001$) based on analysis of variance with the Mann-Whitney test.

Histological observation

According to the examination of the surviving skin tissue near the demarcation between necrotic and survival areas, improvement of angiogenesis in flaps was observed in substance P treatment group demonstrated by significantly higher vessel number (4.6 ± 1.8) compared with the control

group (2.5 ± 1.3) (P=0.006)(Figure 4, Figure 5).

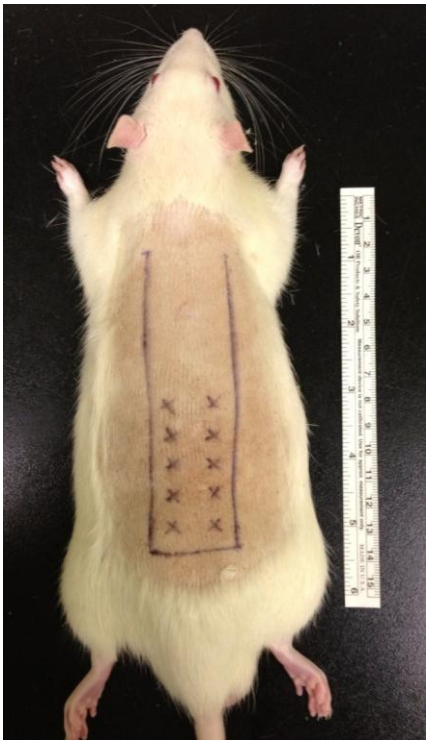

Figure 1. Design of the skin flap. A cranially based 10 × 3cm flap was designed with its base on the lower margin of the scapula. Ten points of injection sites were drawn on distal half of the flap.

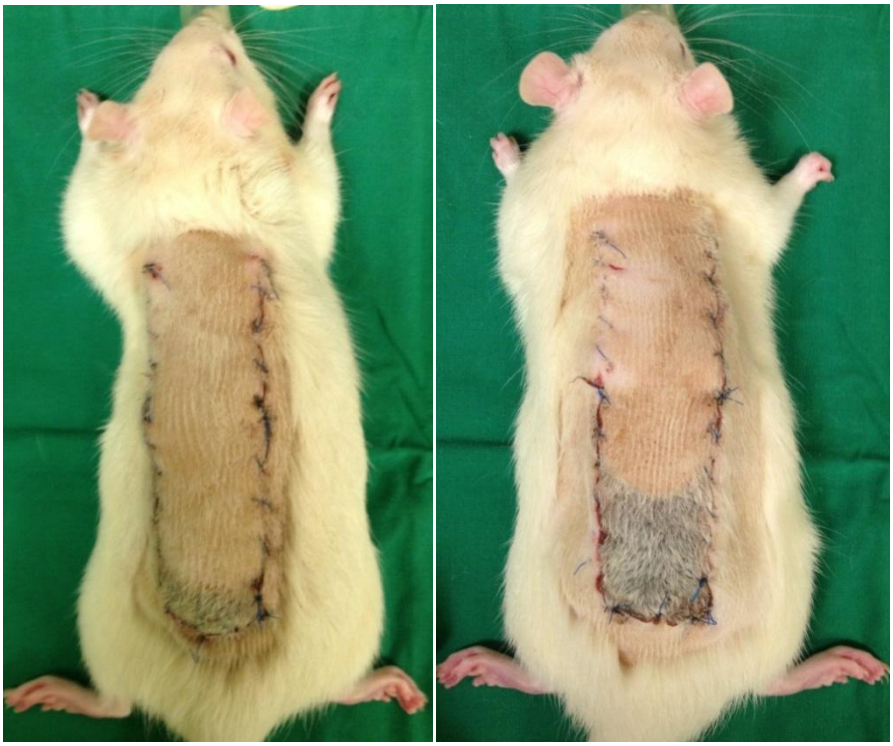

Figure 2. Comparison of survival and necrotic skin flap regions on postoperative day 7 in the substance P treatment group (Left) and control group (Right). The flaps showed clear demarcation between necrotic and survival areas.

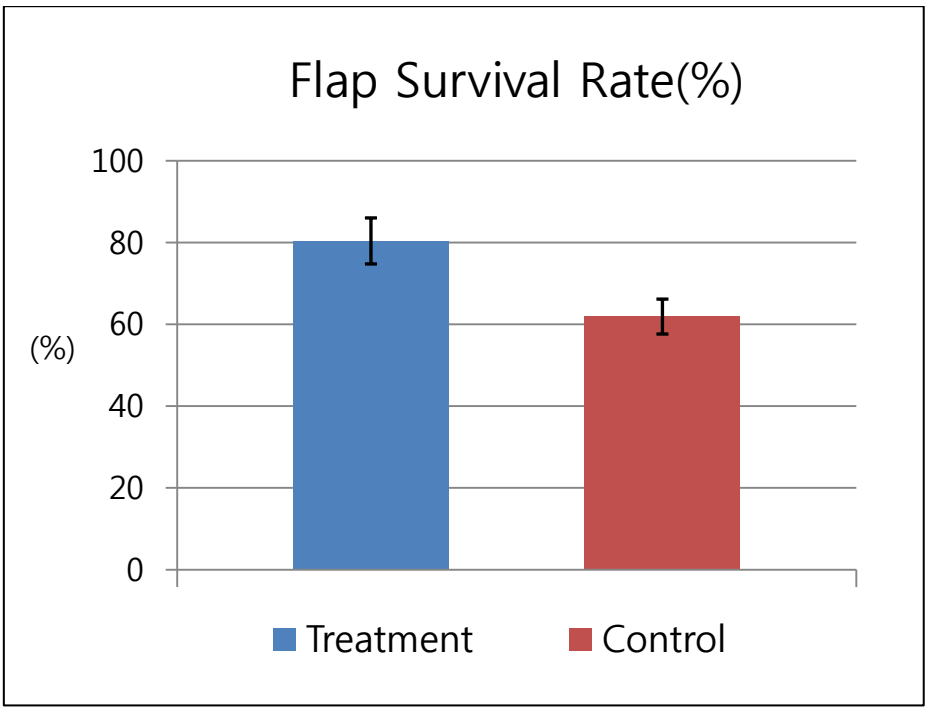

Figure 3. Comparison of mean survival rate of flaps between the two groups on postoperative day 7. The mean percentage of the survival area in the substance P treatment group was 80.4±7.0%, and the mean of the control group was 61.9±6.9%. Substance P increased survival rate of skin flap in rats (***p< 0.001). (Survival rate=Survaival area/Total flap area)

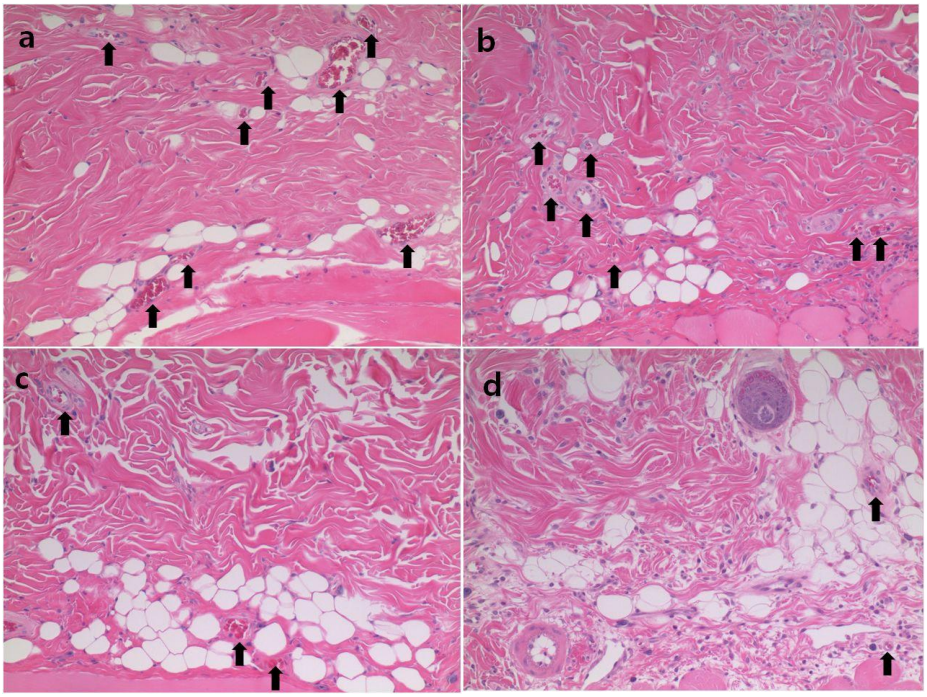

Figure 4. Histological view of the subcutaneous layer of the cross sections (magnification, ×200). Higher vessel number in treatment group (a, b) compared with the control group(c, d). The average number of mature vessels containing erythrocytes(arrows) per site in subcutaneous layer of each flap tissues from the surviving district near the demarcation between survival and necrotic areas was determined.

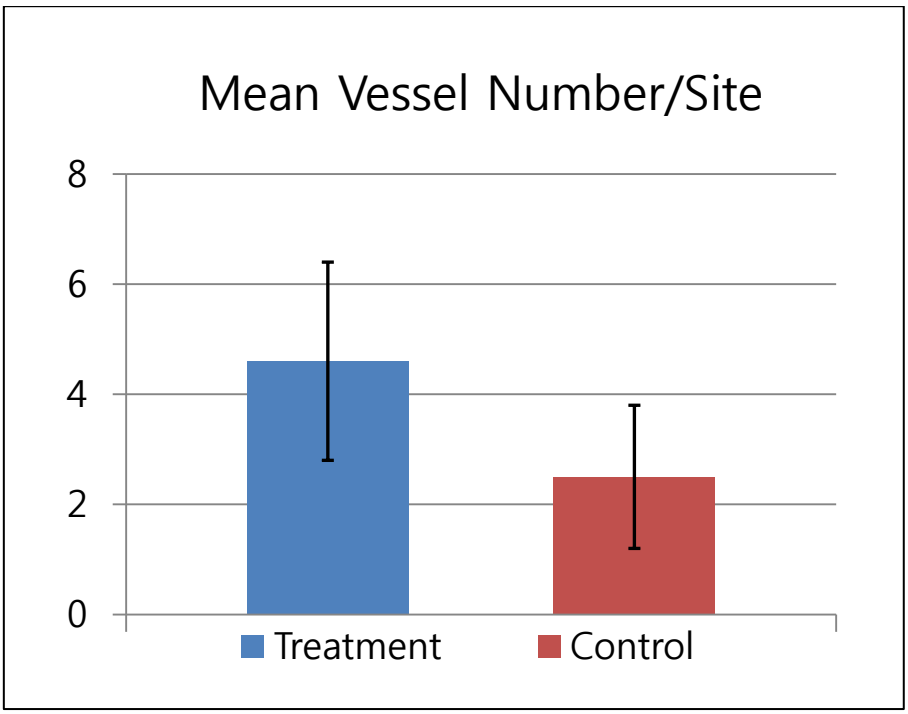

Figure 5. Comparison of vessel number between the two groups. Substance P treatment group demonstrated by significantly higher mean vessel number (4.6 ± 1.8) compared with the control group (2.5 ± 1.3) (p=0.006).

DISCUSSION

Defect coverage using skin flaps is very common in plastic and reconstructive surgery. Adequate vascularity is the most important factor for survival of the flaps, and partial or complete flap necrosis can result from injuries to the vascularity or the inaccurate design of the flap.

Many techniques have been introduced to minimize these problems. The surgical delay method has been used previously for increased viability of the flap, but this method has the disadvantage of requiring two operations. Subsequently, many pharmacological agents have been introduced to limit or reduce ischemia of the skin flap. These agents include sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free radical scavengers. However, most of the agents are for systemic applications and have to be introduced in high doses, and thus some incidence of systemic side effects is a risk. To overcome these disadvantages, topically applied drugs such as nonivamide, nicoboxil and tadalafil have been introduced, but

their effects are limited.

Although a large number of vasoactive-vasodilating agents are reported to possess angiogenic activity[14-17], the relevance of vasodilation to the angiogenesis process is largely unknown.

Endothelium-dependent vasodilation has been clearly demonstrated to be caused by the endothelium-derived relaxing factor identified as nitric oxide (NO)[18 19], which acts at the cellular level by increasing cyclic guanosine monophosphate (GMP)[20].

The tachykinin substance P is released when noxious stimuli activate the peripheral endings of primary sensory neurons, causing vasodilation and increased vascular permeability. The vasorelaxant response to substance P is endothelium dependent and is mediated by NK1 receptors[21 22]. Previous investigations have shown that substance P activates cyclic GMP production in the capillary endothelium[23] and that NO-generating drugs promote endothelial cell proliferation in vitro[24].

The present study evaluated effects of local administration of substance P on viability in rat dorsal skin flap. When the survival area was calculated 7 days after elevation, the flap survival rate was significantly higher in substance P-injected

group (80.4±7.0%) than in the control group (61.9±6.9%) ($p<0.001$). Furthermore, histological observation showed that vessel number of the flap is increased in substance P-injected group. This result indicate that angiogenesis did occur when substance P had been injected.

However, angiogenesis itself cannot explain the benefit of substance P application to the flap. Nitric oxide (NO) seems to play a significant role. NO is the one of the key regulators in tissue perfusion. It is mainly produced in endothelial cells by the endothelial isoform of nitric oxide synthase (eNOS), which has been reported causing endothelium to vasodilate[25].

Previous reports from Ziche et al and others have indicated that substance P, via NK1 receptors, promotes angiogenesis in vivo, endothelial cell growth, and mobilization in vitro. And NO production induced by vasoactive agents, such as substance P, functions as an autocrine regulator of the microvascular events necessary for neovascularizaiton and mediates angiogenesis.

The results of this experimental study showed that substance P, as an effective vasoactive agent, enhances the vessel number of the flap and increases survival rate of skin flaps in rats. However it requires sufficient time and a suitable dose to act as

an angiogenetic factor for increasing flap vascularity and viability before flap elevation.

CONCLUSION

The angiogenic properties of tachykinin neuropeptide substance P, after local administration at the distal half of cranially orientated skin flap in rats, had a clear beneficial effect on the survival of the flap, in a dose of 100nmol. Enhancement of vessel number was obvious at the flap treated with substance P. On the basis of present findings in this study, The author think that substance P may thus represent a therapeutic potential in improving skin flap survival in clinical settings. However, further investigation is needed to better define the optimal dosage and application intervals.

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국문 초록

서론: 피부 피판에서 발생하는 괴사(necrosis)는 재건성형 분야에서 해결해야 하는 과제 중 하나로 주목 받고 있으며 약물의 투여를 통해 피부 피판의 괴사를 줄이고 생존율을 향상시키는 연구들이 광범위하게 진행되고 있는 실정이다. P물질은 말초신경의 말단에서 분비되는 신경전달 물질 중 하나인데 여러 가지 기전을 통해 혈관의 신생(angiogenesis)과 성장을 촉진하는 기능을 발휘하고 있다고 보고 된바 있다. 본 실험의 목적은 P물질의 이러한 기능을 전제로 국소적인 투여를 통해 쥐 피부 피판의 생존율을 높일 수 있는 지에 대해 고찰하고 자 한다.

방법: 총 24마리의 흰 쥐가 실험에 사용되었고 군 당 12마리씩, P물질을 투여하는 실험군과 생리식염수를 투여하는 대조군으로 나누어 실험을 진행하였다. 마취된 쥐의 등에 10 × 3 cm 크기의 피부 피판을 디자인 후, 피판의 먼 쪽(distal) 2분의 1되는 부분에 각각 1ml의 P물질(100nmol)과 같은 양의 생리식염수를 균일하게 피하조직층(subcutaneous layer)으로 주입한다. 다음 디자인 된 피판을 거상(elevation)하고 즉시 원 위치에 다시 봉합시킨다. 봉합 후 7일 째 되는 날, 피부 피판의 생존율을 측정하였고 피판에 대한 조직학적 검사를 시행하였다.

결과: P물질을 투여한 실험군에서 피부 피판의 생존율이

80.4±7.0%로 대조군의 61.9±6.9%에 비해 통계학적으로 유의하게 높아졌음이 관찰되었다($p<0.001$). 조직학적 소견으로는 대조군에 비해 실험군에서 피판의 혈관 개수(vessel number)가 유의하게 증가하였음을 알 수 있었다.

결론: 본 실험의 결과로부터 알 수 있는 바, 쥐에서 P물질을 국소적으로 피부 피판에 투여하면 혈관 개수(vessel number)가 증가하여 피판의 생존율을 제고시킬 수 있다.

주요어: P물질, 피부 피판 생존율, 혈관 신생

학번: 2011-23017