THE EFFECTS OF RETINOIC ACID AND VITAMIN A ON THE VIABILITY OF THE SKIN FLAPS OF THE RATS

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✔ Retinoic acid in the topical application increases vascularity and stimulates mitotic activity in the skin. Vitamin A increases the collagen synthesis, neoangiogenesis, wound contraction and prevents the reperfusion damage, which arises due to the free radicals in the ischemic flap.
In our study, the effects of retinoic acid and vitamin A were researched on increasing the viability of the random pattern caudally pedicled dorsal rat flaps. The viable and non -viable flap areas of the rats- those which were applied topical retinoic acid and those which were applied oral vitamin A- were compared to the rats in the control group, which weren't applied medication, and the results gained were evaluated.
In our study, vitamin A and retinoic acid weren't found useful in increasing the viability of the skin flaps of rats.
Key words: *Vitamin A, retinoic acid, rats, flaps, viability*

✔ **Retinoik Asit ve Vitamin A'nın Ratlarda Deri Flebi Yaşayabilirliği Üzerine Etkisi**
Retinoik asit topikal uygulanımda deride damarlanmayı arttırmakta ve deride mitotik aktiviteyi stimüle etmektedir. Vitamin A kollagen sentezini, neoanjiyogenesisi, yara kontraksiyonunu arttırmakta ve iskemik flepte serbest radikaller ile oluşan reperfüzyon hasarını engellemektedir.
Çalışmamızda retinoik asit ve vitamin A'nın rastgele kanlanan kaudal tabanlı dorsal rat flebinin yaşamsallığını arttırmadaki etkisi araştırılmıştır. Topikal retinoik asit ve oral vitamin A kullanılan ratların yaşayan ve yaşamıyan flep alanları medikasyon verilmeyen kontrol grubu ratlar ile karşılaştırılmış ve alınan sonuçlar değerlendirilmiştir.
Vitamin A ve retinoik asit çalışmamızda ratlarda deri flebi yaşayabilirliğini arttırmada faydalı bulunmamıştır.
Anahtar kelimeler: *Vitamin A, retinoik asit, rat, flep, yaşayabilirlik*

INTRODUCTION

Using flaps is the most often referred operation method for the reconstruction of tissue defects in the plastic and reconstructive surgery and especially in the reconstructive surgery[1,2]. The most important factor in the failure of the reconstructive surgery is the flap necrosis[3]. The aim of the researches for preventing the flap necrosis has been to increase the circulation in flaps. Although many pharmacological agents and physical factors have been tested, still there aren't any methods whose clinical succeses were proved and which are in routine use[4-6].

In topical application, retinoic acid increases vascularity and stimulates the mitotic activity in the skin[7,8]. It is used in the topical treatment of acne vulgaris, in the treatment of the photo-aged skin and the old skin[9-11] In a study on pigs, it was reported that it accelerated the recovery of skin wounds at partial thickness[7]. It was published that it eased re-epithelization after chemical peel and dermabrasion in humans[12,13].

Vitamin A is necessary for the stimulation of fibroplasia, synthesis of collagen and epitelization[14]. The principal effect of vitamin A in the wound recovery is on the collagen syn-

thesis. İt increases the collagen synthesis and the resistance of the wound by influencing the fibroblast cells[15,16]. It effects in such a way as to increase the inflamation process, angiogenesis and wound contraction by its specific effect on macrophages[17].

In this study, the effects of retinoic acid and vitamin A on increasing the viability of flaps was researched and it was thought that it could be useful for the clinical use of flaps.

MATERIAL AND METHOD

In the study, 30 male Spraque-Dawley rats between 200-250 g were used. The rats were divided into three equal groups. The first group was applied oral vitamin A, the second group topical retinoic acid treatments. The third group wasn't given any medication and was evaluated as the control group.

As the rat model, the caudally pedicled, 9x2 cm sized dorsal skin flap was chosen[18,19].

Immediately after anesthetizing by temporary ether anesthetic, they were given 1 mg/kg ketamine intramuscular for the continuation of the anesthesia. After fixing their extremities on a fixed platform, their dorsal areas were shaved in such a way that no bristles should remain in the surgical areas where flaps were planned.

On the dorsal area of the rats, 9x2 cm sized, caudally pedicled, dorsal rat skin flap was drawn.

The flap was dissected in such a way that it should exclude the panniculus carnosus muscle and should be random pattern. After cauterizing the haemorhage points, if any, and spraying 1 cc 10% diluated rifampicine by injector under the flap, the flap was sutured to the dissection area at equal sutural intervals and some collodium solution was spread on its edges. After unfixing them, the rats were taken under control by keeping them alone. The rats which harmed their flaps were let off the study (Figure 1).

Within seven days, ten rats were orally given beta-carotene (vitamin A) at double dose of daily need (4000 I.U./kg/day) in corn oil[20]. Within the same duration, ten rats were topically spread all-trans-retinoic acid (retinoic Acid) at the 0. 025 % mg concentration and in the jel form on their flaps in such a way that it should be a thin film layer. And within the same duration ten other rats were evaluated as the control group and they weren't applied medication.

On the control (seventh) day, the rats were anesthetized by temporary ether and intramuscular ketamine anesthetic. After the fixing process, the viable and non-viable limits of the flaps were drawn and marked by putting polyethylene film (PEF) on them. The viable and non-viable flap areas were calculated by milimetric papers (Figure 2).

The Kruskal Wallis variance analysis and the Mann Whitney U test were applied to compare the findings. The arithmetical averages of the data were issued together with their standard errors.

RESULTS

The viable flap areas of the groups were calculated by using milimetric papers and the percentages of the viable flap areas of each group were put out statistically (Table I).

The average viable flap areas of ten rats which were given vitamin A is 67.00%±6.26

The average viable flap areas of ten rats which were topically applied retinoic acid is 82.33%±2.51.

The average viable flap areas of rats (the control group) which weren't applied medication is 74.9% ±3.16.

Some difference in respect of the viable flap areas between the groups was found statistically ($X^2 = 6.45$, $p<0.05$).

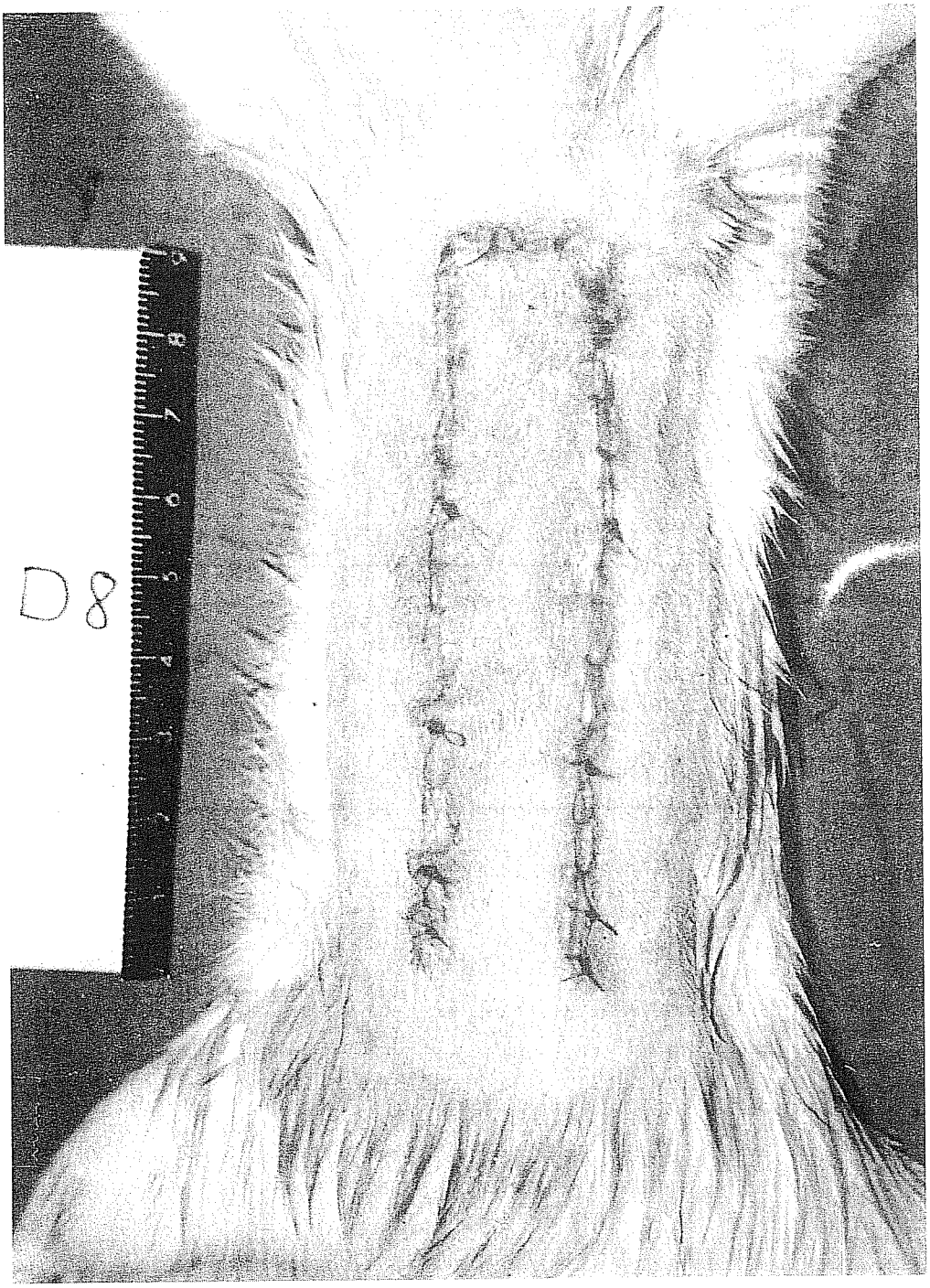

Figure 1. Caudally pedicled, random pattern dorsal skin flap of a rat in the study.

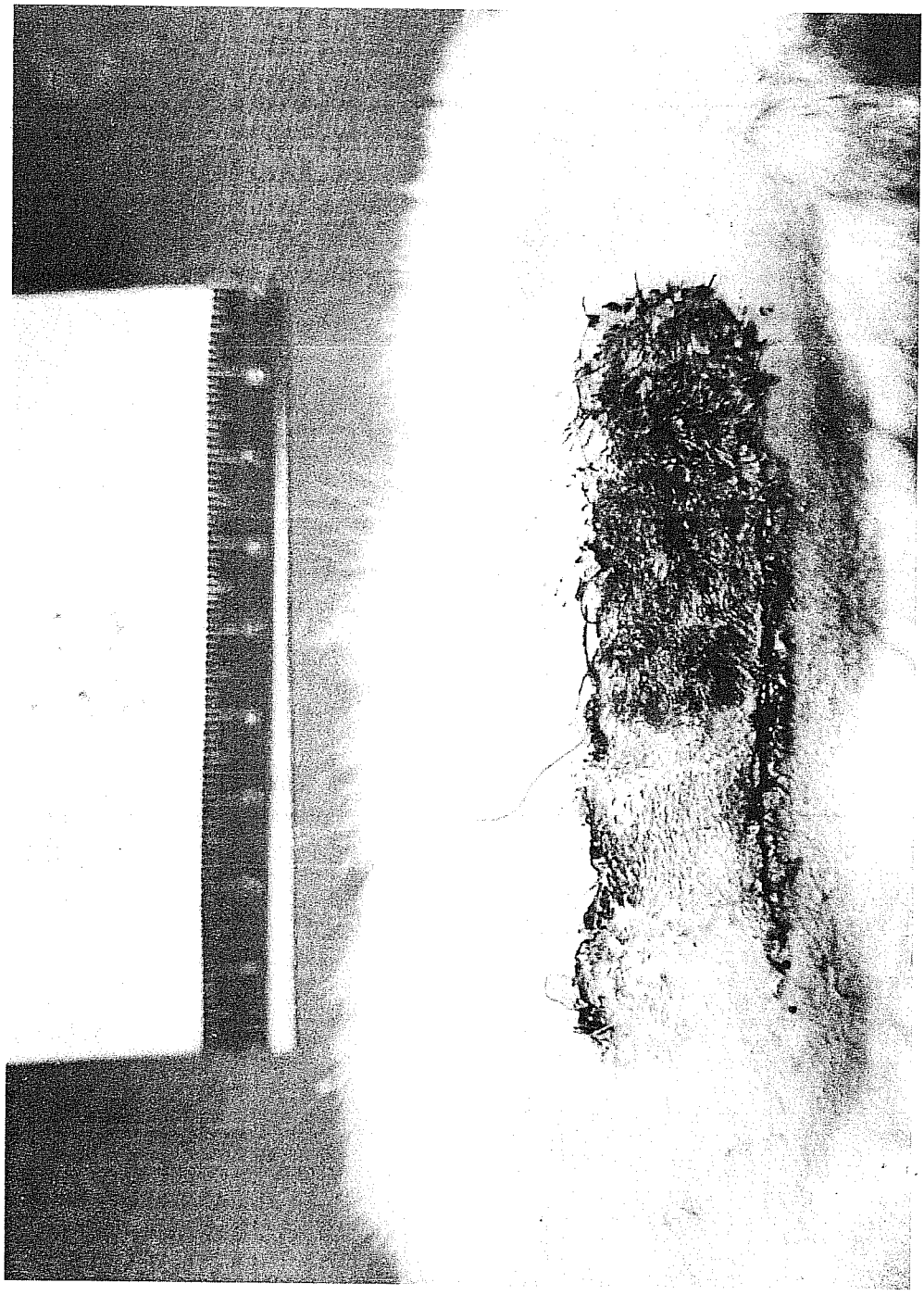

Figure 2. A flap on seventh day.

Table I. Results of Flap Survival Using Vitamin A and Retinoic Acid.

Group	Agent	Dose	Route	N	Mean Survival	SEM
Vitamin A	Beta Carotene	4000 IU/kg	peroral	10	0.67	0.626
Retinoic acid	all trans r.acid	0.025 % mg	topical (jel)	10	0.8233	0.251
Control	no medication	-	-	10	0.749	0.316

The viable flap areas of the rats in the control group and those which were applied retinoic acid were compared and a statistically meaningful difference between the groups wasn't determined ($p>0.05$).

The viable flap areas of the rats which were applied retinoic acid and those which were given vitamin A were compared and a statistically meaningful difference between the groups was determined ($p<0.05$).

DICCUSSION

Visually marking the viable and non-viable flap areas by PEF and the calculation of areas by milimetric papers is a simple and reliable method[16]. The limits of the viable and non-viable flap areas can be demonstrated well. The viable part of the flap has the color and build of the live skin and it can be observed that bristles grow on some of the viable part. On the other hand, the necrotic part of the flap has a dark tone and is sclerotic, and bristles don't come out.

A flap is a wound which heals on a wide surface[2]. The wound is contracted during the healing[14,21]. Measuring the lengths of the viable and non-viable flaps only may put out erroneous results due to flap contraction. Calculating the viable and non-viable flap areas is correct and more reliable in the assessment. In our study, the viable flap areas were assessed.

As a result of a statistical assessment, no meaningful effect by retinoic acid and vitamin A in respect of increasing flap viability was determined.

In the statistical assessment of the average viable flap areas of the rats which were given vitamin A and those which were applied retinoic acid, there is a meaningful difference. However, the result wasn't deemed helpful and useful clinically as there isn't a clinically and statistically meaningful difference between the two groups and the control group.

This result doesn't support our hypothesis that retinoic acid and vitamin A increase flaps' viability.

In a similar study on pigs, it was determined that retinoic acid doesn't increase flaps' viability[22]. The reason shown for that negation were that the retinoic acid treatment increased intercellular oedema and vascularity, although it was hoped that the effective blood current of the flap should increase due to the increase in vascularity, it affected contrarily to what was hoped and the venous blood current, which is as important as the arterial blood current in flaps, caused venous congestion and decreased.

In a similar study on rats, it was determined that vitamin A increases the viability of flaps[23]. In the above mentioned study, vitamin A was used at the dose of 250 mg/kg/day. The effect of increasing flaps' viability of vitamin A was explained through its effect of decreasing free oxygen radicals and protecting the cellular membrane directly.

Vitamin A, used in our study, is only twofold of the daily need of a rat and it was given only for seven days. The hypervitaminotic

dose of vitamin A for rats is much higher than that which we used[20]. Thus, any negative effect isn't subject between the dose of vitamin A and that the viable flap parts of rats are fewer than those of the ones in the control group. The vitamin A dose in the above mentioned study is much higher than our dose (4000 IU/kg/day) and the hypervitaminotic dose for rats and it is 250 mg/kg /day (~450.000 IU/kg/day). Therefore, it is more probable that the dose of vitamin A used in our study is an ineffective dose to increase the viability of flaps.

That the viable flap areas of the rats which were given vitamin A are less than the control group can be clarified by the effect of increasing angiogenesis of vitamin A. It was determined that vitamin A increases the proliferation of endotel cells and the growth speed of the endotel cells of the bovin's aort [24]. Besides , the effects of vitamin A on the wound recovery are similar to retinoic acid's, and it was determined, in the study with retinoic acid, that the increase in vascularity doesn't increase the supply of a flap[22]. Also, it is strongly probable that the effect of increasing angiogenesis by vitamin A causes increase in arterial vascularity in flaps and, therefore, increase in venous vascularity is insufficient.

In the above mentioned study, in which overdose vitamin A was used, it was reported that vitamin A increased the viability of flaps. But, in our study it was determined that vitamin A doesn't increase, on the contrary, it reduces viability of flaps. The two different results can be explained through different effects of vitamin A at different doses on flaps. Probably, vitamin A used at the dose in our study reduces viability of flaps but it increases viability at a high dose.

In our study, vitamin A was used at the dose suitable for clinical use. But, in the above mentioned study, it was used a dose 4.5 times as much as the hypervitaminotic dose, which may cause toxic complications in clinical use.

CONCLUSION

The effects of increasing viability of flaps by oral vitamin A and topical retinoic acid were researced and studied. As a result of the study, vitamin A and retinoic acid weren't found useful in increasing viability of flaps.

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