Journal of the Turkish Academy of Dermatology
eISSN 1307-394X

Research

The Effects of Topical Application of Adapalene and Tretinoin on Tissue Hydroxyproline Content in Wound Model Impaired by Corticosteroid

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Published:
J Turk Acad Dermatol 2011; **5 (1)**: 1151a1.
This article is available from: http://www.jtad.org/2011/1/jtad1151a1.pdf
Key Words: corticosteroid, tretinoin, adapalene, wound healing, hydroxyproline

Abstract

Background: It was well known that inhibitory effect of corticosteroids on cutaneous wound healing antagonized by the use of topical tretinoin and systemic vitamin A. However, the exact mechanism of this antagonistic interaction is not yet fully known. The aim of this study was to investigate the effects of topical tretinoin and adapalene application on tissue hydroxyproline content in corticosteroid impaired full-thickness wound model.

Material and Methods: Forty Wistar Albino rats weighing 200-250 g were divided into five equal groups. A circular full-thickness wound was made for each animal with a standard 8-mm punch biopsy on the midline of the back. No treatment was given to group I (control). Methyl cellulose gel was applied to wound topically once a day in group 2 (placebo). Single dose methyl prednisolon acetate (Depo-Medrol®) 4.5 mg was administered subcutaneously in group III (corticosteroid group), IV and V. The wounds of each rats in group IV and group V were treated with tretinoin 0.1% cream and adapalene 0.1 % gel once a day respectively. Any additional treatment was given to rats in group III. The wound of each animal was excised at the end of the experiment (7th day) and tissue hydroxyproline levels were measured by using spectrophotometric method. Erythema and erosion of skin surrounding the wound were noted as a reaction of irritation.

Results: The mean tissue level of hydroxyproline of the corticosteroid group was significantly lower than all other groups. In group 4 and 5, the mean hydroxyproline tissue levels of the wound were higher when compared with control and placebo groups, and the difference was statistically significant. No any signs of irritant reaction were observed in the treatment group.

Conclusion: The present study demonstrates that methyl prednisolon acetate significantly decreases the tissue hydroxyproline content of full-thickness wound in rats and topical application of adapalene and tretinoin appreciably reverses this inhibitory effect of corticosteroid.

Introduction

Corticosteroids affect almost every phase of wound healing and delay the appearance of inflammatory cells, fibroblasts, and the deposition of ground substance, collagen, regenerating capillaries, contraction, and epithelial migration [1]. Retinoids have long been associated with wound healing. The increasing use of retinoids as pretreatment before epidermal injury, such as laser resurfacing and chemical peeling, is based on its beneficial properties on wound healing [2]. All-trans-retinoic acid, a metabolite of vitamin A, is a potent modulator of cellular proliferation and

cellular differentiation and it has been found to improve healing of partial and full-thickness wounds when applied topically [**3**]. Also, vitamin A deficiency is known to retard the wound healing process. The effects of many retinoids are investigated in experimental and clinical wound healing models and topical tretinoin is one of the first retinoids that has been used in these studies [**4, 5, 6**]. Tretinoin have has also been reported to have the ability to reverse the deleterious effects of corticosteroids on wound healing. Although steroid retardation of wound healing is a significant clinical problem and a commonly used laboratory model in the study of repair processes, little is known about the mechanisms of either steroid retardation or reversal by retinoids [**1, 6, 7, 8, 9, 10**]. Adapalene is a naphtoic acid derivative, synthetic drug with strong retinoid agonist pharmacology and it is known to behave similary similarly to tretinoin pharmacologically [**11**].

The collagen molecule is characterized by the repeating sequence Gly-X-Y, with X often being proline and Y often being hydroxyproline. It is important in all phases of wound healing and is critical to regaining tissue integrity and strength. Hydroxyproline (HP) is the end product of collagen breakdown. Measurement of the tissue hydroxyproline content is one of the significant parameters of the healing process. It could be used as an objective index for collagen production [**12**].

Considering the important role of collagen metabolism in wound healing, it is plausible that the mechanism of retinoid antagonism against deletorius effect of steroid on wound healing may be in part mediated through HP metabolism.

In this study, we tested the hypothesis that steroids and topical application of tretinoin and adapalene have differential effects on tissue HP content and thereby may provide a mechanism by which steroids impair wound healing, and topical retinoids improve this impairment.

Materials and Methods

Forty adult Wistar-albino rats weighing between 200-250 g were used. Guidelines for using laboratory animals were strictly followed throughout the study. The local Ethics Committee at the Suleyman Demirel University approved our study protocol. The animals were acclimatized for one week to our laboratory conditions prior to experimental manipulation. They had free access to standard laboratory chow and water ad libitum.

Experimental Setting

Anesthesia was induced with intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine (5 mg/kg). After shaving, a circular, full thickness wound was made for each animal under sterile conditions with a standard 8-mm dermatologic punch biopsy, on the midline of the back. The wounds were than sutured with silk 4/0. The animals were sacrificed on the seventh postoperative day and the wounds on the back were excised for biochemical analyses.

The animals were randomly assigned into five groups each containing eight animals.

Group 1: was served as control group.

Group 2: (placebo group) Methyl cellulose %10 was administered topically to the wound

Group 3: 4.5 mg methyl prednisolone acetate was applied subcutaneously.

Group 4: Subcutaneous 4.5 mg methyl prednisolone acetate and topical tretinoin %0.1 to the wound were administered.

Group 5: Subcutaneous 4.5 mg methyl prednisolone acetate and topical adapalene %0.1 to the wound were administered.

Treatment groups were compared with control, placebo and corticosteroid group according to the tissue HP level. At the end of the seven days, all animals were examined for signs of skin irritation.

Biochemical Analyses

The tissue samples taken for hydroxyproline determination were washed with physiological saline and dried for 72 h. in an etuve adjusted to 100°C. HP levels were measured spectrophotometrically in dry tissue modifying the method described by Woessner [13] after samples were weighed and hydrolyzed in concentrated hydrochloric acid (HCl) at 130°C for 3 h. After each sample was adjusted to a final volume of 1 mL, samples were centrifuged at 3000g for 15 min to obtain supernatant. A second centrifugation at 2500g for 5-10 min was performed after isopropanol addition to an equal volume of supernatant.

Statistical Analyses

Data were expressed as mean and standard error of the mean (SEM). Analyses of variance and Kruskal-Wallis tests were used in the comparison of HP levels of groups and $P<0.05$ considered as statistically significant.

Results

In control, placebo, corticosteroid, topical tretinoin and topical adapelene groups, mean HP levels were measured as: 92,9 ± 13,7 µg/mg, 88,1 ± 16,5 µg/mg, 28,3±9,3 µg/mg, 104,1 ± 5,8 µg/mg, and 96,2±6,5 µg/mg respectively (**Table 1**). The HP content of the group 3 was significantly lower than all other groups ($p<0.01$). There were no significant differences in HP levels between group 4 and group 5 ($p>0.05$), but on the other hand, HP levels of these groups were significantly higher when compared with control and placebo groups ($p<0.05$, $p<0.05$ respectively). No signs of irritant reaction were observed in the treatment group.

Discussion

Corticosteroids are widely used in the treatment of several kinds of diseases for their anti-inflammatory and immunosuppressive functions. In addition to therapeutic function, their adverse effects became evident and impaired wound healing during corticosteroid therapy had become a serious clinical problem. Among the factors implied for the impaired wound healing, corticosteroids are commonly investigated and their harmful effects are reported in various tissues [**1, 3, 7**].

Retinoids have long been associated with wound healing, but its mechanism of action has not been fully elucidated. Various impaired animal wound models have shown vitamin A to exert beneficial effects through promoting deposition of collagen, stimulating fibroblast activation, neovascularization, elastin formation and upregulation of the plasminogen activator system [**1, 3, 5**].

In contrast, some study results about the influence of vitamin A derivates on wounds have been controversial. *Popp* et al. [**14**] found the healing rate of full-thickness wounds in humans to be accelerated by topical tretinoin. *Kitano* et al. [**15**] reported that pretreatment with all-trans-retinoic acid reversed impaired wound healing in diabetic mice. *Basak* et al. [**16**] reported enhancement of collagen production, angiogenesis and granulation tissue formation under tretinoin treatment. On the other hand, *Hung* [**6**] showed that tretinoin application retarded wound healing and reepithelialization possibly through persistent dermal inflammation. *Oikarinen* et al. [**17**] found that tretinoin decreased procollagen synthesis in a dose-dependant manner. *Dzubow* et al. [**18**] demonstrated no improved healing of full-thickness wounds after a 2-month course of retinoic acid. *Golan* et al. [**8**] could not detect an improvement in wound healing with the topical use of vitamin A. In another animal study reported by *Watcher* et al. [**19**], postoperative application of tretinoin to open lesions was reported to result in a significant retardation of reepithelialization. Also, a significant increase of dietary vitamin A has been shown to yield no beneficial effects on wound healing [**20**].

Adapalene is a synthetic topical retinoid which is known to behave similarly to tretinoin pharmacologically and it has more stable and less irritating formulation [**11**]. There is limited studies related to effects of

Table 1. Hydroxyproline Level of all Groups

	Rat No	Group 1	Group 2	Group 3	Group 4	Group 5
HP Levels (µg/mg)	1	91,7	64,5	39,1	111,3	102,5
	2	79,1	96,5	37,3	99,4	101,3
	3	69,3	66,9	35,2	98,2	93,2
	4	104,5	90,1	17,7	102,1	95,0
	5	112,1	83,8	33,3	105,0	99,4
	6	96,1	106,3	19,9	102,6	103,5
	7	100,5	109,0	15,6	114,5	90,3
	8	90,0	87,5	28,6	100,1	84,8
	Mean±SE	92,9±13,7	88,1±16,5	28,3±9,3*	104,1±5,8**	96,2±6,5**

SE: Standart Error; * p<0,05 vs other all groups; ** p<0,05 vs Groups 1 and 2; p>0,05 between Groups 4 and 5.

topical adapalene application on wound healing process. *Basak* et al. [**16**] performed a study on the effects of tretinoin, adapalene in an experimental model of wound healing. They excised the wounds for biochemical examination with HP levels on the seventh and 14th day. A significant decrease in HP levels was detected at day 7 and an increase at day 14 in the tretinoin group. HP results revealed no difference either in the adapalene or in the collagenase group vs. the control at day 7 or 14.

Retinoids have been also reported to have the ability to reverse the deleterious effects of corticosteroids. Their beneficial effects on wound healing impaired by corticosteroids were demonstrated on various tissues. The exact mechanism of retinoid antagonism against the effect of corticosteroids in wound healing cascade is unclear [**1,7, 9**]. Since *Erlich* and *Hunt* [**9**] first demonstrated in 1968 that vitamin A stimulated wound healing impaired by glucocorticoid hormones, many possible mechanism for these antagonistic effects have been proposed [**1, 2, 21, 23**]. *Uland* et al. [**23**] reported that vitamin A exert beneficial effects through disinhibiting the depressed arginine to ornithine metabolism. It has been postulated that the predicted suppressive effects of steroids and the stimulatory effects of retinoids on wound healing process are related to expression of growth factors including transforming growth factor beta (TGF-β) and insulin-like growth factor-1(IGF-I) [**1**]. *Phillips* et al. [**24**] found that corticosteroids significantly impaired the healing of small and large intestine anastomoses, with decreased bursting pressures at 1 week and high dose retinol therapy reversed the inhibitory effects of corticosteroids.

In conclusion, enhancement of the HP levels on wound impaired by corticosteroid with the application of tretinoin and adapalene suggested that they contributed to wound healing through increasing collagen production. The results indicated that adapalene and tretinoin promoted tissue HP content significantly and was able to overcome the wound healing-suppressing action of corticosteroid in rat model.

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