Effects of Tretinoin Pretreatment on TCA Chemical Peel in Guinea Pig Skin

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This study was done to characterize the structural changes in the tretinoin pretreatment on trichloroacetic acid(TCA) chemical peel. In guinea pigs, the right halves pretreated with tretinoin and the left halves treated nothing were compared in their structural changes after TCA chemical peel. Epidermal thickness in the tretinoin pretreated group was almost the same in the first and second week. But epidermis of the TCA group increased continuously. In the first week, mitotic figures in the epidermis were more increased in the TCA group, but those in hair follicles were more increased in the tretinoin pretreated group. In the second week, mitotic figures in the epidermis were almost same in both group, but in hair follicles of the tretinoin pretreated group, mitotic figures were much more increased. In alcian blue staining, glycosaminoglycan was stained more strongly in dermis of the TCA group in first week, but was more strongly stained in the tretinoin pretreated group in second week. On electron microscopic findings, the fibroblasts in upper dermis were larger and had plentier cytoplasm with more organelles in the tretinoin pretreated group. Conclusively, tretinoin pretreatment on TCA chemical peel sustained the effects of TCA longer and showed synergistic effects of TCA and induced enhanced wound healing.

Key Words : Tretinoin, Trichloroacetic acid, Chemical peel

INTRODUCTION

Trichloroacetic acid(TCA) chemical peel has been used to treat actinic keratosis, solar elastosis and wrinkles(Monash,1945; Ayres,1960). Several kinds of techniques have been applied for TCA chemical peel(Van Scott and Yu,1984; Brody and Hailey,1986; Letessier, 1989). Recently, tretinoin pretreatment in TCA chemical peel is one of the most popular techniques for promoting TCA effect and prevention of postinflammatory hyperpigmentation(Brody and Hailey,1986; Resnik,1989).

Tretinoin or all-trans retinoic acid is a synthetic vitamin A analogue used in topical therapy of several skin diseases(Kligman et al.,1986; Weiss et al.,1988). It also promotes wound healing and accelerates collagen synthesis in papillary dermis(Hermann and Woodward,1972; Prutkin,1972; Dlein,1975; Hung et al.,1989; Kim et al., 1995) and improves pigmentary abnormalities including melasma and postinflammatory hyperpigmentation(Kligman and Willis,1975; Barbara et al,1981; Pathak et al., 1986).

There were few reports about the effects of tretinoin pretreatment on the percutaneous absorption of TCA (Mandy,1986; Resnik,1989; Hevia et al.,1991). However, the mechanism of tretinoin pretreatment on TCA chemical peel were not so clear because many studies were done on clinical parts(Mandy,1986; Hevia et al.,1991).

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The purpose of this study is assess the mechanisms for the effects of tretinoin pretreatment in TCA chemical peel on guinea pig skin through structural changes.

MATERIALS AND METHODS

Materials

Eight mature male guinea pigs(supplied from Korea University Animal Laboratory)weighing about 300 grams were used. Tretinoin(Stieva A[®] solution 0.05%) were kindly gifts from Stiefel Canada Inc. The TCA solution was prepared as 35g of TCA crystal(Janssen chimica, BP, USP) in 100ml of distilled water. And we used the 35% TCA solution as a medium-depth wounding agent in guinea pig skin according to the result of previous report(Kim CG et al., 1994).

Methods

Tretinoin pretreatment and sampling :

Eight guinea pigs were divided into control group and experimental group. After shaving the hair off the dorsal skin of the guinea pigs, the right halves of dorsal skins of control and experimental group were painted with 0.05% tretinoin solution for 2 weeks and left halves of dorsal skins of control and experimental group were not pretreated. On day 15, the chemical peel was done on the both sides of dorsal skins of experimental group with 35% TCA solution.

The TCA solution was applied uniformly with cotton-tipped applicators until the skin turned white(frosting) and then the frosting was neutralized with normal saline. So the control group were further divided into tretinoin pretreated group and no-pretreated group. We used the tretinoin pretreated group as control group. And the experimental group were further divided into TCA treated group, and tretinoin and TCA combined group. After 1 week and 2 weeks, both sides of the dorsal skins were taken by exision. Halves of those specimens were soaked into the 10% formalin solution for various stains and the remaining halves were soaked into the 2.5% gluteraldehyde solution for electron microscope.

The effects on epidermal thickness :

The epidermal thickness from the base of the stratum corneum to the basement membrane was measured on the 7 sites of each histologic section by optical micrometer(eyepiece reticle) under 100 power light microscope. The measurements were done with two anatomical pathologists seperately and the results

were analyzed by statistically. And we also observed the thickness of each layer on each histologic section under 400 power light microscope.

The number of mitotic figures :

The mitotic figures in the epidermis and hair follicles were counted. The grid was inserted over the ocular lens in a 400 power light microscope and the total mitotic figures per grid area were counted in 10 area of each section on hematoxylin and eosin stains. Two anatomical pathologists count the mitotic figures separately and the results were analyzed by statistically.

Dermal changes :

The dermal changes were observed by Mason's trichrome stain for collagen, elastic stain for elastin and the Alcian blue stain for glycosaminoglycan.

Electron microscopic examination :

Changes of fibroblasts on upper dermis were observed.

Statistics

The mean and the standard deviations were calculated, and the differences of the mean between the experimental groups were judged by ANOVA and the analysis of the results between the experimental groups were judged by Duncan's and Tukey's multiple range test.

RESULTS

The thickness of epidermis

The changes of total epidermal thickness: The control group showed the decrease of epidermal thickness according to the tretinoin pretreated duration. So the mean epidermal thickness of control group in first week is 0.061 mm but that in second week is 0.034 mm. The mean epidermal thickness was 0.077 mm(126% of control) in TCA treated group and 0.112 mm(184% of control) in the tretinoin and the TCA combined group in the first week. On second week, the mean of epidermal thickness was 0.087(256% of control) mm and 0.060 (176% of control) in each group(Table 1, Figure 1).

The changes of each layer of epidermis: The stratum corneum and stratum spinosum of epidermis in TCA treated group and the stratum graulosum of epidermis in TCA combined group were thicker than control group in the first week. The stratum spinosum of epidermis in

Table 1. Mean epidermal thickness(mm)* (H & E stain, X 100).

	First week (%)	s. d.	Second week (%)	s. d.
Control group	0.061(100)	0.012	0.034(100)	0.004
TCA treated group	0.077(126.2)	0.014	0.087(255.9)	0.018
Tretinoin and TCA combined group	0.112(183.6)	0.018	0.060(176.6)	0.009

* Values are mean epidermal thickness of 7 sites of each histologic section per unit length.

* All differences are significant at $p < 0.0001$

Table 2. Total numbers of mitotic figures in epidermis and hair follicles on 10 grid areas(H & E stain, X 400).

	Control group		TCA treated group		Tretinoin and TCA combined group	
	Epidermis	Hair follicle	Epidermis	Hair follicle	Epidermis	Hair follicle
First week	3	1	12	4	6	14
Second week	3	3	1	14	2	28

Fig. 1. Light microscopic findings of epidermal thickness in the control(left), TCA treated(middle), tretinoin and the TCA combined(right) groups at 2 weeks showed the thickest epidermis in the TCA treated group(H & E stain, $\times 200$).

TCA treated group were thicker than control group in the second week(Fig. 1).

The number of mitotic figures

The numbers of epidermal mitotic figures were 3 in the control group in the first and second weeks. But the numbers of mitotic figures in hair follicles were increased from 1 to 3 in the second week. The numbers of epidermal mitotic figures were 12 in the TCA treated group and 6 in the tretinoin and TCA combined group in the first week. After the second week, the numbers of epidermal mitotic figures were 1 and 2 in each group. The numbers of mitotic figures in hair follicles were 4 in TCA treated group, and 14 in tretinoin and

TCA combined group in the first week. After the second week, The numbers of mitotic figures in hair follicles were 14 and 28 in each group(Table 2, Fig. 2).

Dermal changes

On collagen and elastic stains there was no significant differences between the TCA treated and the tretinoin and the TCA combined group.

On Alcian blue at pH 2.5 stain for glycosaminoglycan, the TCA treated and the tretinoin and the TCA combined group were stained more strongly than the control group in the upper dermis. In the TCA treated group, specimens from the second week were stained more strongly than in those of first week. The TCA

Fig. 2. Light microscopic findings of hair follicles in control(left), TCA treated(middle), tretinoin and TCA combined(right) groups at 2 weeks showed increased numbers of mitotic figures in both treated groups(H & E stain, $\times 400$).

Fig. 3. Light microscopic findings of dermal glycosaminoglycans in control(left), TCA treated(middle), tretinoin and TCA combined(right) groups at 2 weeks showed the strongest staining reaction in tretinoin and TCA treated group(Alcian blue stain, $\times 100$).

treated group was stained strongly compared with tretinoin and the TCA combined group on the first week. But after the second week, the tretinoin and the TCA treated group were stained more strongly(Fig. 3).

Electron microscopic findings

In the tretinoin and the TCA combined group compared with the control group, fibroblasts shows the active state with larger and more cytoplasm filled with many organelles(Fig. 4, 5).

DISCUSSION

Trichloroacetic acid(TCA) is a chemical cauterant that coagulates the proteins of the skin. It chemically destroys the epidermis and upper dermis, and the skin is replaced by new epidermis that migrate from the cutaneous adnexa(Stegman, 1980, 1982; Nelson et al., 1995). Because of the tretinoin effects on rapid wound healing, change of skin pigmentation, many clinicians used tretinoin before and after TCA chemical peeling,

Fig. 4. Electron microscopic photograph of fibroblasts in the upper dermis of guinea pig skin at 2 weeks($\times 3500$). A, Control group. The Cell in the center is an inactive fibrocyte with shrunken cytoplasm and few organelles. B, TCA treated group. C, Tretinoin & TCA combined treated group. This is a large fibroblast, with extensive cytoplasm crowded with increased amount of rough endoplasmic reticulum.

Fig. 5. Higher magnification of electron microscopic photograph of Figure 4($\times 7000$). A, Control group. B, TCA treated group. C, tretinoin & TCA combined treated group. In the tretinoin and TCA combined group, fibroblasts were larger and had plentier cytoplasm with many organelles.

dermabrasion and laserabrasion. Hevia et al(1991) showed that tretinoin pretreatment in TCA chemical peeling significantly enhanced healing. However, his study was done on clinical parts and he could not show the underlying changes.

Our study was done to characterize the structural changes in the tretinoin pretreatment on TCA chemical peel. And the morphological changes of epidermis and dermis were assessed.

There were many possible explanations for tretinoin

pretreatment on wound healing. It has marked effects on epidermal cell production, desquamation and dermal connective tissue(Stegman,1982;Hevia et al.,1991). Also tretinoin increase the epidermal mitotic activities(Zil, 1972; Lee et al.,1973; Plewig and Braun-Falco,1975). Zil(1972) reported that peak of epidermal hypertrophy was induced after 1 week of tretinoin application and after 2 weeks, reversion of the hypertrophy toward normal occurs, with a steady-state equilibrium persisting indefinitely.

According to our results about the changes of epidermal thickness, i.e., the tretinoin and TCA combined group showed relatively a little changes than the TCA treated group between first week and 2 weeks, and the changes of mitotic figures of the epidermis and hair follicles, i.e., the tretinoin and TCA combined group showed more early appearance and more increased numbers of mitotic figures in hair follicles than the TCA treated group in first and second weeks, tretinoin pretreatment induced the effects of TCA very early and sustained the effects of TCA for a longer time. We thought in case of tretinoin pretreatment, epidermis repaired by their own reproduction at first but later, epidermal repair was through the differentiation of hair follicles.

Tretinoin increased the collagen synthesis (Beach and Kenney, 1983; Kim et al., 1995) and decreases collagenase production (Abergel and Meeker, 1985). Also, TCA cause replacement of the epidermis but also produces long-lasting histologic changes in the dermis (Stegman, 1982). In our study, the changes of collagen and elastic tissue on upper dermis were almost the same in tretinoin and TCA combined and TCA treated group. But electron microscopic findings showed differences in the fibroblasts of upper dermis of both group. In the tretinoin and TCA combined group, fibroblasts were larger and had plentier cytoplasm with many organelles. That means fibroblasts in tretinoin and TCA combined group were more activated than that of TCA treated group.

Deposition of glycosaminoglycan or mucin-like substance has been seen in the epidermis after topical and systemic retinoid treatment (Kligman et al., 1986) but the dermal glycosaminoglycan-like substance was absent following treatment with topical tretinoin (Weiss and Ellis, 1988). In our study, glycosaminoglycan was deposited a little bit more in the dermis of the TCA treated group at first week. But in the second week, glycosaminoglycan was deposited more in the tretinoin pretreated group. So, we think that tretinoin pretreatment prolonged the effects of TCA on the upper dermis.

Conclusively, tretinoin pretreatment had synergistic effects on TCA, induced enhanced wound healing and prolonged the effects of TCA longer time.

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