# International Journal of Dermatology © 1999 Blackwell Science Ltd.

Volume 38(2)

February 1999

pp 149-153

Tretinoin-iontophoresis in atrophic acne scars [Pharmacology and Therapeutics]

Schmidt, Jolanta B. MD; Donath, Peter MD; Hannes, Johanna MD; Perl, Sylvia MD; Neumayer, Renate MD; Reiner, Angelika MD

From the Department of Dermatology, Division of Special and Environmental Dermatology and Institute of Clinical Pathology, University of Vienna Medical School, Vienna, Austria Correspondence: Jolanta B. Schmidt, MD; Department of Dermatology; University of Vienna Medical School; Währinger Gürtel 18-20; A-1090 Vienna; Austria

PayPerView Account PayPerView Cart

Full Text LinkBrowse Search ResultsFull Text Link

Titles Display Main Search Page Full Text LinkBrowse Table of ContentsFull Text Link

TOC

#### Outline

- Abstract
- Patients and methods
  - Patients
  - Iontophoresis
  - Clinical documentation
  - Histologic documentation
- Results
  - Clinical evaluation
  - Histology and immunohistochemistry
  - Side-effects
- Discussion
- References

### Graphics

- Table 1
- Figure 1
- Table 2
- Table 3
- Table 4

# Output... Print Preview Email Article Save Article Links... Full Text Abstract Complete Reference Help Logoff History... Tretinoin-iontophoresis i... Go Previous Page

# Abstract

Background: Atrophic acne scars are a frequent problem after acne. Hitherto, mainly invasive treatment measures were possible. In a recent paper, we demonstrated the positive effects of iontophoresis with 0.025% tretinoin gel vs. estriol 0.03%.

Objective: In this further study, the recording of the clinical effects of iontophoresis with 0.025% tretinoin gel in atrophic acne scars was supplemented by immunohistochemistry investigations of collagen I and III, proliferation markers, and the estimation of epidermal thickness.

Methods: The treatment was performed twice weekly in 32 volunteer patients for a period of 3 months by application of the substance under a constant direct current of 3 mA for 20 min. Skin biopsies prior to and at the end of treatment were performed in 32 voluntary patients in order to investigate collagen I/III and proliferation markers by immunohistochemistry methods.

<u>Results</u>: Clinically, at the end of treatment, in 94% of patients a significant decrease in the scar depth was observed. Neither epidermal thickness nor proliferation markers revealed a significant increase at the end of treatment. Furthermore, collagen I and collagen III showed no common trend, as expressed statistically by a lack of significance. In some cases, increases in collagen III became evident at the end of treatment.

Conclusions: Tretinoin-iontophoresis is an effective, noninvasive treatment of atrophic acne scars without causing disturbing side-effects.

Acne scars, a widespread problem, represent persisting impairments for patients. While acne keloids are attributed to a genetic disposition, possibly due to an increased response of fibroblasts towards androgenic stimulation, the cause of the development of atrophic or icepack scars, mostly developing spontaneously, still remains unclear. Patients suffering from multiple atrophic acne scars feel disfigured and are continuously reminded of their previous disease.

Hitherto, the following therapeutic methods were available in order to improve atrophic acne scars. Collagen augmentation is limited to single acne scars and results in only moderate, nonpersisting effects. For multiple scars, dermabrasion can be performed with good success. Moreover, chemopeels and lasers are therapeutic alternatives. The latter methods are invasive, and leave the patients with a crusted face at least for several days.

We have developed a new method without any intermediate disfigurement for the patients. In an earlier study, iontophoresis was performed in 46 patients with atrophic acne scars with either 0.1% estriol in aqueous solution or 0.025% tretinoin gel. The treatment showed good clinical results in 93% of patients in the tretinoin group; 1 in a post-treatment survey of 2 years, these results were found to be permanent. In the present study, the clinical findings of iontophoresis treatment with tretinoin gel were supplemented by immunohistochemistry investigations of collagen I and III distribution, proliferation markers, and measurements of skin thickness. Positive effects of tretinoin cream in acne scarring have been described, 2 and were attributed to the influence of the substance on fibroblast function.

Patients and methods Patients

Thirty two patients aged from 15 to 48 years (mean, 25) were treated by iontophoresis with 0.025% tretinoin gel. The therapy was performed in 19 women with a mean age of 25 years (15-39) and in 13 men with a mean age of 26 years (18-48).

Iontophoresis

Iontophoresis is well known in physical medicine, whereby antiinflammatory substances penetrate into

tissues, such as muscles or joints, thus achieving higher local tissue levels but avoiding systemic side-effects.3 Our previous findings with estriol iontophoresis had supported the local effect of iontophoresis treatment in adequate concentrations and frequency due to stable estrogen levels during treatment. Using a constant, direct current of, in our case, 3 mA, polar substances diffused along a diffusion gradient between anode and cathode. Thus, changes in the electric cell membrane properties are achieved, with a subsequently increasing intracellular accumulation of the substance, 3 which seems to be released in a prolonged way from a depot.

For the treatment, tretinoin gel was applied to the cleaned face and covered by a thin cotton cloth. Thereafter the iontophoresis mask-a metal facial mask covered by a sponge material and with openings for nostrils and mouth-was placed on the face. Under the influence of galvanic current, acid solutions diffused from the cathode towards the anode. The instrument used was a MINISAN low-frequency instrument (Dr. Schuhfried Medizintechnik, Vienna, Austria). Iontophoresis with tretinoin gel was performed twice weekly for 20 min over a period of 3 months.

## Clinical documentation

Treatment effects were clinically documented by recording (subjective) assessments of scar depths, skin firmness and elasticity, pore size, and skin moisture according to the parameters: minor, clear, and significant findings. For this purpose, we used a personal evaluation scheme (Table 1) that was applied for each of the parameters. Changes in effects were only recorded if observed by both monitor and patient. Photographs for clinical documentation were taken at the start and at the end of treatment.

Graphic

Help with image viewing Table 1 Evaluation of clinical effects of treatment on the atrophic scars

# Histologic documentation

For the investigation of the epidermal and dermal effects of tretinoin-iontophoresis, epidermal thickness and proliferation markers, as well as collagen I and collagen III, were estimated. The collagen parameters were also of special interest in comparison with the mode of action of estrogen treatment on skin, where significant collagen III and procollagen III increases were reported at the end of treatment and paralleled the clinical effects in aging skin.4,5

Comparable clinical effects between estriol- and tretinoin-iontophoresis in our previous study raised the question of whether influences on collagen, comparable with those achieved with topical estriol, were a possible mode of action for tretinoin-iontophoresis.

In all of the 32 voluntary patients, who had provided written informed consent, skin was obtained in order to perform histology and immunohistochemistry prior to and at the end of the treatment period. Under local anesthesia with 2% Scandicain, two punch biopsy specimens (3 mm in diameter) were taken from the atrophic scar within the treatment areas on the face. Skin biopsies were formalin fixed and paraffin embedded. Histologic sections were prepared according to routine histologic techniques and stained with hematoxylin and eosin.

Collagen I distribution was investigated using a rabbit anti-human collagen polyclonal antibody (Chemicon Int. Inc., Temecula, CA, diluted 1:800).

For the detection of collagen III, a rabbit polyclonal antibody (Monosan, The Netherlands, diluted 1:10) was used.

For the immunohistochemical staining of collagen fibres, slides were enzyme pretreated with 0.1% protease type IV (Sigma, St Louis, USA) for 10 min. The slides were then incubated with the primary antibody for 1 h at room temperature; further staining was performed according to the immunohistochemical ABC-method.

For the determination of proliferating cell fractions, we used MIB1 (Clone MIB1, Dianova GmbH, Hamburg, Germany, diluted 1:50), a mouse monoclonal antibody directed against the proliferation associated Ki-67 antigen, which can be used on formalin-fixed, paraffin-embedded tissues. Paraffin sections were incubated in citrate buffer (pH 6) in the microwave oven, first at 120 W for 10 min and then three times for 5 min at 450 W. Citrate buffer was refilled several times in order to keep the slides moist. The slides were cooled for 15-20 min at room temperature and, after incubation with the primary antibody for 1 h, they were further processed using the immunohistochemical ABC-method.

For all immunohistochemical staining procedures, products from Vector Laboratories (Burlingame, CA) were used. Briefly, after incubation with the primary antibody and incubation with a biotinylated secondary antibody, incubation with the ABC-complex for 1 h each followed. The reaction product was developed with diaminobenzidine-tetrahydrochloride. Finally, slides were counterstained with Harris' hematoxylin. All incubation steps were carried out at room temperature.

Collagen I and III staining was estimated semiquantitatively; the staining intensity was classified as weak (+), intermediate (++), or strong (+++).

The MIB1 labeling index was obtained by counting a minimum of 1000 cells of the epidermal layer with a  $1 \text{ cm}^2$  ocular grid under  $400 \times$  magnification and calculating the percentage of positive cell nuclei. Nuclei showing even brown staining were regarded as positive.

The thickness of the epidermal layer was measured using a measuring ocular (Leitz) which shows a hundred unit scale. The scale was calibrated using a glass slide carrying a 2-mm scale divided into units of 0.01 mm each (Leitz). For calibration, both scales were adjusted in parallel side by side and matched; the length of one unit of the measuring ocular scale was then determined. Measurements were carried out using  $400 \times 10^{-5}$  magnification. The result obtained in units of the measuring ocular was multiplied by 0.0032.

Results Clinical evaluation

The clinical effects of treatment showed improvement by a decrease in scar depth in 94% of patients (Fig. 1). Only in two patients was no decrease in scarring observed according to subjective patient opinion, the investigator's clinical evaluation (Tables 1 and 2) and the photographs. In both men and women the first effects on the scars were noted after 8 weeks of treatment and increased from then on. The subjective assessments of the skin showed improvements of skin firmness and of pore size diminution in 47% and 55% respectively after a mean of 9 weeks. Skin moisture revealed no common trend during the treatment period. Both increases (16%) and decreases (38%) were observed.

Graphic
[Help with image viewing]

Figure 1 Atrophic acne scars. (a) Before treatment. (b) After 3 months of tretinoin-iontophoresis.

Graphic
[Help with image viewing]

Table 2 Clinical effects at the end of treatment

Histology and immunohistochemistry

Neither epidermal thickness nor the proliferation markers (MIB1) revealed significant differences between initial and final values (Table 3).

Graphic Help with image viewing

Table 3 Mean values of skin thickness and proliferation markers during tretinoiniontophoresis

With regard to the collagen parameters (collagen I and III), no common trend became evident at the end of treatment. Equal staining, decreases and increases were noted. Stable amounts of collagen I were found in 47% and in 25% for collagen III. Increases and decreases in collagen I were observed in 25% and 28% respectively. For collagen III, increases and decreases were both 37.5% (Table 4).

Graphic

[Help with image viewing] Table 4 Collagen I/III during tretinoin-iontophoresis

Side-effects

Flushing of the treated skin occasionally persisted for 30 min after treatment, and was interpreted as a physiologic response due to the increased microcirculation. Dryness of the skin was reported in 32% of cases and was accompanied by fine scaling in four cases.

Discussion

Iontophoresis with tretinoin was shown to be an efficient therapy for atrophic acne scars. The present, clearly positive findings confirm the preliminary results of our previous study.

Interpretation of the positive clinical effects was sought by evaluation of some of the epidermal parameters, such as MIB1 and epidermal thickness. Although our data conflict with studies that describe increases in epidermal thickness by tretinoin in aging skin 6-8 and increases in epidermal mitotic indices,9 it must be kept in mind that these studies were performed in aged, elastotic skin. In acne scars of more juvenile skin, both parameters may not be responsible for the clinical benefits of treatment. Furthermore, the report of different effects of tretinoin at various stages of treatment 10 could serve as a possible explanation for the lack of significant effects of tretinoin on epidermal properties of acne scars at the end of treatment.

According to the investigated collagen parameters, it was shown that collagenous tissue and mainly collagen III represent an initial point of action in some cases. Increases in collagen stimulation 11,12 and improvements in elastic fiber properties 13 have been reported with tretinoin, and are combined with an increase in the number of keratinocytes 14 responsible for the effects of tretinoin in acne and in skin aging. The various modes of action of the substance have been extensively investigated in both conditions. Moreover, striae distensae 15 and acne scars 2 also respond positively to topical tretinoin.

The loss of collagen fibers and the degradation of elastic fibers are found in photodamaged skin, 13 and thus are comparable to some extent with the histologic features observed in atrophic scars. The stimulatory effects of tretinoin on collagen synthesis 4 and on increases in fibroblast collagen 16 may be considered as the theoretical background of the possible action of tretinoin on acne scarring. In our investigation, immunohistochemistry revealed collagen III changes by tretinoin-iontophoresis. It is beyond the scope of this

study to determine the mechanisms by which tretinoin-iontophoresis exerts its effect. This will be the subject of further investigations.

In the present study, the clinical effects of tretinoiniontophoresis, as confirmed by post-treatment controls after 6 and 12 months, were quite impressive and, as reported previously, permanent. This observation definitely excludes edema as a possible mechanism for the decrease in depth of the scars. Preliminary experience with the effects of iontophoresis with estrogen in skin aging in comparison with those of topical cream treatment in identical concentration 17 have shown that the clinical efficacy of iontophoresis treatment is significantly superior to that of daily treatment with cream only.

A 3-month treatment period with iontophoresis with tretinoin gel is therefore not comparable with topical application of a cream of the same concentration for the same time period. By iontophoresis, increased tissue concentrations are achieved, and the properties of cellular membranes and the intracellular uptake of substances are influenced in an as yet undetermined manner. Although an important part of treatment in physical therapy, dermatologists have not paid adequate attention to this method until recently. 18

According to the "flip-flop" gating model-one proposed mechanism for the mode of action of iontophoresis-pore formation in the stratum corneum is induced by iontophoretic currents. Pores are formed between neighboring keratin helices as a result of molecular realignment and repulsion of neighboring dipoles. 19 Hair follicles and sweat gland ducts act as diffusion shunts, offering paths of reduced resistance for iontophoretic transport. 20 The explanation of the extent and persistence of the effects of treatment is therefore not only due to the effects of tretinoin alone, but also to those of iontophoresis itself.

The method of iontophoresis should therefore be investigated further. Up to now, in dermatology, only tap water iontophoresis has been widely used for the treatment of hyperhidrosis. 21

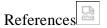
According to the previous 1 and present findings, iontophoresis can be used in order to improve the effects of substances in comparison with those achieved by topical application of compounds of the same concentration. With changing conditions, e.g. increased tretinoin concentration or increased frequency of treatment, the systemic action of tretinoin cannot be excluded and thus blood determinations are required. These should be performed with regard to the teratogenicity of the substance. Although no experimental data are available on the magnitude of the increase in concentration in skin tissue after iontophoresis, we conclude from the grade and persistence of the improvements that the above-mentioned mechanisms 3 contribute to depot formation in the skin.

Our first report on the positive effects of tretinoin-iontophoresis in acne scars has been corroborated by the clinical findings and photographs in the present study. The mode of action is not clear. Immunohistochemistry data conflict to some extent with previous findings on tretinoin effects in solar atrophy. Further investigations should therefore be performed with regard to the cellular effects of iontophoresis.

Clinically, iontophoresis with tretinoin gel was confirmed to improve acne scars effectively without any intermediate impairment of the appearance of the patients. According to the present findings, iontophoresis itself seems to be a promising method for enhancing the effects of a substance, in our case tretinoin.

One explanation for the positive clinical effects may be the initial point of action of tretinoin on skin collagen.

Further explanations for the extent of improvement should involve an investigation of the method of iontophoresis.



1 Schmidt JB, Binder M, Macheiner W, et al. New treatment of acne scars by iontophoresis with estriol and

- 2 Harris DW, Buckley CC, Ostlere LS, et al. Topical retinoic acid in the treatment of fine acne scarring. Br J Dermatol 1991; 125: 81-82 (letter). [Context Link]
- 3 Pratzel HG, Macheus R, Dittrich P. Iontophorese zur forcierten Hautresorption von iontomethacin und Salicylsäure. Rheumatol 1986; 45: 74-78. [Context Link]
- 4 Varila A, Rantala I, Oikarinen A, et al. The effects of topical oestradiol on skin collagen of postmenopausal women. Br J Dermatol 1995; 102: 985-989. [Context Link]
- 5 Schmidt JB, Binder M, Demschik G, et al. Treatment of skin aging with topical estrogens. Int J Dermatol 1996; 35: 669-674. Ovid Full Text Bibliographic Links [Context Link]
- 6 Kligman AM, Grove GL, Hirose R, et al. Topical tretinoin for photoaged skin. J Am Acad Dermatol 1986; 15: 836-859. Bibliographic Links [Context Link]
- 7 Lever L, Kumar P, Marks R. Topial retinoic acid for treatment of solar damage. Br J Dermatol 1990; 122: 91-98. <u>Bibliographic Links</u> [Context Link]
- 8 Weiss JS, Ellis CN, Headington JT, et al. Topical tretinoin improves photoaged skin. A double-blind vehicle-controlled study J Am Med Assoc 1988; 259: 527-532. [Context Link]
- 9 Kligman AM. Guideline for the use of topical tretinoin (Retin A) for photoaged skin. J Am Acad Dermatol 1989; 21: 650-654. Bibliographic Links [Context Link]
- 10 Yamamoto O, Bhawan J, Hara M, et al. Keratinocyte degeneration in human facial skin: documentation of new ultrastructural markers for photodamage and their improvement during topical tretinoin treatment. Exp Dermatol 1995; 4: 9-19. Bibliographic Links [Context Link]
- 11 Schilz JR, Lanigan W, Nabial B. Retinoic acid induces cyclic changes in epidermal thickness and dermal collagen and glycosaminoglycan biosynthesis rates. J Invest Dermatol 1986; 87: 663-667. <u>Bibliographic Links [Context Link]</u>
- 12 Kim RY, Stern WH. Retinoids and butyrate modulate fibroblast growth and collagen matrices. Invest Ophthalmol Vis Sci 1990; 31: 1183-1186. <u>Bibliographic Links [Context Link]</u>
- 13 Noble S, Wagstaff A. Tretinoin. A review of its pharmacological properties and clinical efficacy in the topical treatment of photoaged skin Drugs Aging 1995; 6: 479-496. [Context Link]
- 14 Tong PH, Horowitz LA, Wheller LA. Transretinoic acid ehances the growth responses of epidermal keratinocytes to epidermal growth factor and transforming growth factor beta. J Invest Dermatol 1990; 94: 126-131. Bibliographic Links [Context Link]
- 15 Elson ML. Topical tretinoin in the treatment of striae distensae and in the promotion of wound healing: a review. J Dermatol Treat 1994; 5: 163-165. [Context Link]
- 16 Panizzon RG, Bruckner-Tuderman L, Mindek G, et al. The influence of tretinoin on photodamaged human skin: a biochemical, cytogenetic and histological evaluation over 9 months. Dermatology 1994; 189: 336. [Context Link]
- 17 Schmidt JB. Topica estrogen treatment of skin aging and acne scars. Clinical effects and hormone parameters. Progress in Cosmetic Dermatology: Science and Safety. IV. International Meeting on Cosmetic

Dermatology 30.10.-2.11.1991, Rome. [Context Link]

- 18 Kassan DG, Lynch AM, Stiller MJ. Physical enhancement of dermatologic drug delivery: iontophoresis and phonophoresis JAMA 1996; 34: 657-666. [Context Link]
- 19 Phipps JB, Padmanabhan RU, Lattin GA. Iontophoretic delivery of model inorganic and drug ions. J Pharm Sci 1989; 78: 365-369. Bibliographic Links [Context Link]
- 20 Burnette RR, Marrero D. Comparison between the iontopheretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin. J Pharm Sci 1986; 75: 738-743. <u>Bibliographic Links</u> [Context Link]

21 Levit F. Treatment of hyperhidrosis by tapwater iontophoresis. Cutis 1980; 31: 173-175. [Context Link]

Accession Number: 00004342-199902000-00019

Full Text LinkBrowse Search ResultsFull Text Link

Titles Display Main Search Page Full Text LinkBrowse Table of ContentsFull Text Link

TOC

Copyright (c) 2000-2003 Ovid Technologies, Inc. Version: rel9.0.0, SourceID 1.8300.1.161