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Clinical study of a retinoic acid-loaded microneedle patch for seborrheic keratosis or senile lentigo

Sachiko Hirobe [a], Risa Otsuka [a], Hiroshi Iioka [b], Ying-Shu Quan [c], Fumio Kamiyama [c], Hideo Asada [b], Naoki Okada [a,*], Shinsaku Nakagawa [a,*]

[a] Laboratory of Biotechnology and Therapeutics, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Department of Dermatology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634-8522, Japan
[c] CosMED Pharmaceutical Co. Ltd., 32 Higashikujokawanishi-cho, Minami-ku, Kyoto 601-8014, Japan

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ABSTRACT

Aims: Pigmented lesions such as of seborrheic keratosis and senile lentigo, which are commonly seen on skin of people > 50 years of age, are considered unattractive and disfiguring because of their negative psychological impact. Drug therapy using all-trans retinoic acid (ATRA) is an attractive option for self-treatment at home. We have developed an ATRA-loaded microneedle patch (ATRA-MN) and confirmed the pharmacological effects of ATRA-MN application in mice. Here, we describe a clinical study to evaluate the safety and efficacy of ATRA-MN in subjects with seborrheic keratosis or senile lentigo.
Main methods: ATRA-MN was applied to the lesion site of each subject for 6 h once per week for 4 weeks. The skin irritation reaction was scored to assess adverse reactions and blood tests were performed to evaluate the presence of systemic adverse reactions. To assess the treatment effect using ATRA-MN, the desquamation and whitening ability of the investigational skin was observed.
Key findings: Desquamation of the stratum corneum was observed following four ATRA-MN applications at 1-week intervals, but ATRA-MN applications did not induce severe local or systemic adverse effects.
Significance: These results showed that ATRA-MN treatment is promising as a safe and effective therapy for seborrheic keratosis and senile lentigo.

1. Introduction

Seborrheic keratosis and senile lentigo are characterized by pigmented lesions that are commonly seen on skin of people > 50 years of age [1–3]. Seborrheic keratosis is characterized as sharply demarcated brownish plaques with verrucous surfaces. Originating from keratinocytes, they can develop anywhere on the skin, except the palms and soles, and may become irritated and itchy. Senile lentigo appears on sun-exposed areas of the skin such as the face and back of the hands. These lesions are considered unattractive and disfiguring and may have negative psychological impacts. Thus, they are often removed for cosmetic reasons.

Cryotherapy and laser surgery are common treatments for seborrheic keratosis and senile lentigo. Cryosurgery using liquid nitrogen is the standard and most widely practiced treatment for seborrheic keratosis. Another surgical option is laser ablation using erbium YAG or CO_2 lasers [4,5]. However, these treatments incur complications such as scarring, hyperpigmentation, and recurrence. Additionally, because they require specialized equipments, patients must undergo regular outpatient treatments for complete removal of the lesions, incurring high costs.

Drug therapy for seborrheic keratosis and senile lentigo is an attractive treatment option for self-treatment at home but is not well established. Retinoids are natural and synthetic metabolites and analogs of vitamin A and are important regulators of epidermal proliferation and differentiation [6,7]. All-trans retinoic acid (ATRA), a natural retinoid, is the major biologically active form of retinoids. ATRA induces heparin-binding epidermal growth factor-like growth factor (HB-EGF) expression in keratinocytes. HB-EGF can bind to the epidermal growth factor receptor (EGFR) to play an important role in re-epithelialization by increasing keratinocyte proliferation [8]. ATRA treatment leads to epidermal hyperplasia via keratinocyte-derived HB-EGF [6]. Thus, ATRA increases basal keratinocyte proliferation, inducing the accelerated turnover of epidermal cells, leading indirectly to epidermal thickening [9]. These effects could be beneficial in seborrheic keratosis and senile lentigo treatments.

ATRA has some drawbacks such as its poor water solubility and photostability, and skin irritation reactions limit its topical use [10,11]. Furthermore, its skin permeability is relatively low [12]. To overcome these disadvantages, we have developed an ATRA-loaded microneedle patch (ATRA-MN) [13]. Microneedles [14,15] made of dissolving

* Corresponding authors.
E-mail addresses: okada@phs.osaka-u.ac.jp (N. Okada), nakagawa@phs.osaka-u.ac.jp (S. Nakagawa).

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polymers can puncture the stratum corneum (the physical barrier to material permeation), dissolve in water in the skin, and directly deliver ATRA to the epidermis. Previously, we reported that ATRA-MN application induced HB-EGF expression in the skin and epidermal hyperplasia and shortened the stratum corneum turnover time in mice [13]. We also confirmed that an ATRA-MN application potentially will not cause serious adverse events in humans [13].

We conducted a clinical study to evaluate the safety and efficacy of a novel treatment method using ATRA-MN for seborrheic keratosis and senile lentigo.

2. Materials and methods

2.1. ATRA-MN preparation

As described previously [14–19], ATRA-MN was prepared at CosMED Pharmaceutical Co. Ltd. (Kyoto, Japan) using micromolding technologies with sodium hyaluronate as the base material. To form the transcutaneous patch system, ATRA-MN with an area of 0.8 cm^2 (containing 200 microneedles) was affixed to an adhesive film with 2.3 cm^2 area. The length of the microneedles on ATRA-MN was 800 μm. The amount of ATRA loaded into the microneedles was 1.6 μg, using a high-performance liquid chromatography method as reported previously [15,20].

2.2. Clinical study protocol

Eight patients volunteered for and were enrolled in the study (Table 1). Written informed consent was received before enrollment. All procedures in humans were performed at Nara Medical University in accordance with a protocol (Table 2) approved by the ethics committee of the Nara Medical University. ATRA-MN was applied to the lesion site of each subject for 6 h once per week for 4 weeks. The investigated skin was observed every week, and the skin irritation reaction was scored according to the classification of the International Contact Dermatitis Research Group (ICDRG) [21] system (Table 3) to assess adverse reactions. A general blood test and biochemical tests of the liver and renal function were performed before the first and after the last application to evaluate the presence of systemic adverse reactions. To assess the treatment effect using ATRA-MN, the desquamation and whitening ability of the investigational skin was observed.

3. Results

3.1. Safety assessment of ATRA-MN applications

The primary aim of this study was to demonstrate that multiple ATRA-MN applications are safe in humans. Faint erythema, classified as ?+ by the ICDRG scale, was observed as the application number increased (Fig. 1), whereas positive reactions were not induced by ATRA-MN applications. This faint erythema was a temporary reaction that disappeared completely after 3 months. Administration of ATRA-MN did not induce detectable adverse effects, as determined by a general peripheral blood test and biochemical tests of liver and renal function.

Table 1
Patients' information in clinical study.

Factor	Group	Number of subjects
Race	Asian	8
Gender	Male	5
	Female	3
Age (years)	51–60	1
	61–70	2
	71–80	5
Lesion	Senile lentigo	6
	Seborrheic keratosis	2

Table 2
Schedule of clinical study for assessing the safety and efficacy of the ATRA-MN.

Day	0	7	14	21	28	//	111
ATRA-MN application	•	•	•	•			
Skin observation		•	•	•	•		•
Blood test	•				•		

Thus, multiple ATRA-MN applications did not induce local or systemic adverse effects, indicating that ATRA-MN could be safely administered to humans.

3.2. Efficacy assessment of ATRA-MN applications

Having confirmed the safety of multiple ATRA-MN applications in humans, we next evaluated the efficacy of the novel treatment method for seborrheic keratosis and senile lentigo. On the investigational skin, desquamations of the stratum corneum were observed in three subjects after three ATRA-MN applications (Fig. 2). After four applications, the stratum corneum of an additional subject also desquamated. These desquamations were inferred to be caused by acceleration of stratum corneum turnover, resulting from the pharmacological effect of ATRA delivered into the skin. However, a clear skin-whitening effect and desquamation of the seborrheic keratosis lesion skin were not observed during ATRA-MN applications or 3 months after four applications.

4. Discussion

In this study, we applied ATRA-MN four times at 1-week intervals. The pharmacological effect of ATRA was confirmed in humans, but these results showed that this novel treatment method using ATRA-MN needed improvement for the complete cure of seborrheic keratosis and senile lentigo.

The first point is the ATRA dose. In a study, when 0.025% ATRA gel was applied at 5 mg gel/cm^2 (1.25 μg ATRA/cm^2), the dose of ATRA delivered to the epidermis was 20% (0.25 μg ATRA/cm^2) after 40 h [22]. It is estimated that the dose of ATRA delivered to the epidermis is 1–4 μg/cm^2 in 0.1%–0.4% ATRA used in Japan [23]. Therefore, we estimated that a safe delivery dose to the epidermis would be a few micrograms, and in fact, we used 1.6 μg/0.8 cm^2/patch (2 μg/cm^2). In a study of intradermal ATRA injection, a maximum of 5 mL of 0.2 mg/mL ATRA solution was administered to a subject [24]. Although the number of administration sites was unclear, we assumed that 1 mg ATRA was the maximum dose administered to a subject. We would be able to increase the ATRA dose to at least 25 μg/patch while ensuring safe ATRA application.

Moreover, we propose that the negative result is because of the small number of applications and short-term treatment. Although we have no data about ATRA clearance in the skin, we have shown that antigens delivered by MN into mouse skin remained over 48 h [15]. We consider the possibility that the clearance of ATRA with hyaluronic acid, which is a high molecular polymer and a component of MN, is slow. Because the primary endpoint in this study was the safety of the approach, we performed ATRA-MN application once per week, after

Table 3
Scoring of skin local reactions according to ICDRG.

Score	Reactions
−	Negative reaction
?+	Doubtful reaction; faint erythema only
+	Weak (non-vesicular) positive reaction; erythema, infiltration and possibly papules
++	Strong (vesicular) positive reaction; erythema, infiltration, papules, vesicles
+++	Extreme positive reaction; bullous reaction
IR	Irritant reaction