

Antibacterial Activity of Retinaldehyde against *Propionibacterium acnes*

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Key Words

Topical retinaldehyde • *Propionibacterium acnes*

Abstract

Background: Retinaldehyde has been shown to exert antibacterial activity in vitro. **Aim:** This study evaluates the effect of retinaldehyde on *Propionibacterium acnes* both in vivo and in vitro. **Methods:** Microbial minimal inhibitory concentrations (MICs) of retinaldehyde and retinoic acid were determined on reference strains of *P. acnes*. In vivo activity of daily topical application of 0.05% retinaldehyde on the *P. acnes* density was evaluated after application in a single-blind randomised study. **Results:** MICs of retinaldehyde were 4 mg/l for *P. acnes* No. CIP179 and CIP53119 and 8 mg/l for *P. acnes* No. CIP53117. In contrast, the MICs of retinoic acid were superior to 128 mg/l for these three strains. In vivo, retinaldehyde-treated areas displayed a significant decrease in counts of viable *P. acnes* as compared with the untreated areas with a median decrease of 10^2 log *P. acnes*/cm² after 2 weeks of daily application. Vehicle alone had no effect. **Conclusion:** The MIC of retinaldehyde against *P. acnes* suggests a direct antibacterial activity. Daily topical application of 0.05% retinaldehyde is associated with a clear reduction of the *P. acnes* density.

Introduction

Historically, vitamin A or retinol has been considered as an anti-infectious agent [1]. Retinol induces non-specific resistance to infection [2–5], but the mechanisms of these anti-infectious activities remain mostly hypothetical and are probably related to its pleiotropic effects on the immune function [6]. Retinaldehyde is a natural metabolite of retinol, and previous pilot studies have suggested that it has antibacterial activity [7]. Since retinaldehyde is used as a cosmetic ingredient on the face, a zone where *Propionibacterium acnes* is very abundant [8], we wondered if retinaldehyde has a direct action against *P. acnes*. To evaluate this possible antibacterial effect, in vitro and in vivo assays were performed. Minimal inhibitory concentrations (MICs) of retinaldehyde and retinoic acid were determined on reference strains and compared with each other. The in vivo impact of retinaldehyde on *P. acnes* populations was evaluated on the face of volunteers in a single-blind randomised study.

Material and Methods

Reagents

All-*trans*-retinal (Sigma, St. Louis, Mo., USA) was dissolved in a solution of a synthetic triglyceride, silicone and butylhydroxytoluene 0.001% and kept under argon atmosphere at 4°C for the in vivo study. This preparation does not contain any conservative compound which can interact with the *P. acnes* culture, the final retinaldehyde concentration was 0.05%.

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Table 1. MICs (mg/l) of retinaldehyde and retinoic acid against reference strains

Strains	No.	Retinaldehyde	Retinoic acid
<i>Propionibacterium acnes</i>	CIP 179	4	>128
<i>Propionibacterium acnes</i>	CIP 53119	4	>128
<i>Propionibacterium acnes</i>	CIP 53117	8	>128
<i>Escherichia coli</i>	NCTC 10418	>128	>128
<i>Pseudomonas aeruginosa</i>	ATCC 9027	>128	>128

For the in vitro study, retinaldehyde or retinoic acid (Hoffmann-La Roche, Basel, Switzerland) was dissolved in a solution of 50% polyethylene glycol 400 (Sigma) and ethanol (Merck).

Strains

Reference strains were obtained from the American Type Culture Collections (ATCC), Rockville, USA, from the National Collection of Type Culture (NCTC), Calindale, London, UK, and the Collection Institut Pasteur (CIP), Paris, France. These strains were frozen at -20°C in peptone water (BioMérieux, Lyon, France) with 10% glycerol (Sigma). Clinical strains were identified according to conventional identification methods.

In vitro Testing

Microbial MICs were determined by a microdilution method [9] using an inoculum of 10^5 – 10^6 CFU/ml, 'medium 20' as growth medium and an incubation time of 3 days in anaerobiosis (Generbag CO_2 system, BioMérieux). The MIC was defined as the lowest concentration yielding no growth visible to the naked eye.

In vivo Protocol

After randomisation of 22 volunteers, approximately 4 μg of either retinaldehyde with vehicle or vehicle alone were applied daily on a 4-cm² lateral area of the forehead with a small sterile cotton. The other side of the forehead was left untreated. On day 15, skin flora was sampled on both sides and counts of viable bacteria were performed. Skin bacteria were collected by the cylinder scrub method described by Williamsom and Kligman [10]. Paired results from treated (retinaldehyde or vehicle) and untreated areas were analysed using the Wilcoxon rank sum test. The study has been approved by the institutional ethical committee.

Results

MIC assays were made on *P. acnes* reference strains (table 1). MICs of retinaldehyde were 4 mg/l for *P. acnes* No. CIP179 and CIP53119 and 8 mg/l for *P. acnes* No. CIP53117. In contrast, the MICs were superior to 128 mg/l with retinoic acid for these three strains. No activity was found with retinaldehyde and retinoic acid against gram-negative *Pseudomonas aeruginosa* ATCC 8027 and *Escherichia coli* NCTC 10418.

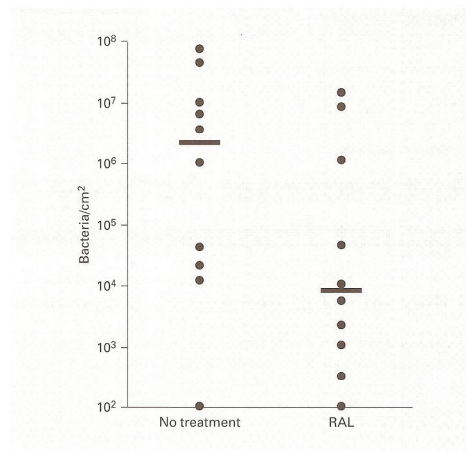


Fig. 1. *P. acnes* density after daily topical application of retinaldehyde (RAL) 0.05% during 2 weeks. Horizontal bars indicate the median values.

The daily application of 0.05% retinaldehyde in the experimental vehicle was well tolerated. Twenty-one of the 22 volunteers were evaluated, 1 volunteer was excluded because of lack of compliance. In each volunteer, counts of viable bacteria from treated areas (retinaldehyde in vehicle or vehicle alone) and untreated areas were compared.

In the group treated with retinaldehyde, the treated areas displayed a significant decrease in the counts for viable *P. acnes* as compared to the untreated areas (fig. 1). Of the 10 volunteers of this group, the *P. acnes* density was reduced in 8, stable in 1 and slightly increased in 1. The median decrease in *P. acnes* density was 10^3 log.

Treatment with the vehicle without retinaldehyde was evaluated in 11 volunteers; the *P. acnes* density did not change. The median density in the area treated with vehicle and the untreated area were 10^7 *P. acnes*/cm² on both sides. This result means that no vehicle effect was found. The difference of *P. acnes* density between the vehicle and the retinaldehyde treatment was statistically significant ($p = 0.048$).

Discussion

In the past, clinical investigations have demonstrated the efficacy of synthetic retinoids in dermatoses in the patho-

genesis of which bacterial agents are implicated such as acne [11] and gram-negative folliculitis [12]. In patients treated with oral 13-*cis*-retinoic acid profound alterations of the skin flora are observed: reduced recovery of *P. acnes* and gram-negative bacteria, increased nasal colonisation by *Staphylococcus aureus* and high incidence of staphylococcal cutaneous infections [13]. However, in vitro experiments with 13-*cis*-retinoic acid [14–16] and acitretin [16] failed to reveal any direct antibacterial activities against either gram-positive (*S. aureus*, *Staphylococcus epidermidis* and *P. acnes*) or gram-negative bacteria. Since retinoic acid does not inhibit bacterial growth by itself [16, this study], changes in skin bacteria likely result from indirect mechanisms, such as reduction of the skin lipid or drying effects. The density of *P. acnes* on the skin depends, among other

factors, mainly on the amount of sebum [17]. The reduction of skin lipids induced by oral 13-*cis*-retinoic acid is probably the main cause for the modification of the *P. acnes* population.

The results obtained with retinaldehyde were different. The decrease of *P. acnes* densities observed in vivo can be partially explained by the retinoid activity of retinaldehyde, but in vitro results suggest a second phenomenon. MICs against *P. acnes* were between 4 and 8 mg/l for retinaldehyde and more than 128 mg/l for retinoic acid. The in vitro contact of retinaldehyde with *P. acnes* inhibited its proliferation, whereas this was not observed with retinoic acid. The mechanisms remain to be determined, but these results suggest a direct antibacterial activity of retinaldehyde against *P. acnes*.

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