Susceptibility of Propionibacterium acnes isolated from patients with acne vulgaris to zinc ascorbate and antibiotics

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Introduction

This study pits various types of vitamin C against one another in inhibiting the growth of a popular acne causing bacterium,then it compares it against various anti-acne drugs.

Conclusions

Zinc ascorbate is the most effective of the vitamin C types for combating acne, as done here.

Zinc ascorbate can be used to great effectiveness with some anti-acne drugs and inhibits the action of other anti-acne drugs.

Amendments

These are "in vitro" (in cells) studies, so making definitive claims may not pan out in normal day to day living.

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ORIGINAL RESEARCH

Susceptibility of *Propionibacterium acnes* isolated from patients with acne vulgaris to zinc ascorbate and antibiotics

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Purpose: The in vitro antimicrobial activity of ascorbic acid derivatives against *Propionibacterium acnes* was tested either alone or in combination with a variety of antimicrobial agents, and their fractional inhibitory concentration index was determined using checkerboard tests. The antimicrobial effectiveness of zinc ascorbate in the treatment of acne vulgaris, either alone or in combination with antibiotics such as clindamycin that are commonly used in Japan for the treatment of acne vulgaris, was therefore examined.

Materials and methods: The antimicrobial susceptibility of 41 strains of clindamycin-sensitive and/or clindamycin-resistant *P. acnes* isolated from acne vulgaris patients was tested, in comparison with a type strain of *P. acnes*.

Results: Zinc ascorbate showed antimicrobial activity against a type strain of *P. acnes* and its concentration (0.064%) was sufficiently lower than the normal dose (5%) of other ascorbic acid derivatives. Combinations of zinc ascorbate with clindamycin, erythromycin, and chloramphenicol showed an additive effect, and zinc ascorbate alone effectively inhibited the growth of all *P. acnes* including clindamycin-resistant strains.

Conclusion: The results provide novel evidence that the combination of zinc ascorbate and clindamycin is effective for acne vulgaris treatment.

Keywords: antimicrobial susceptibility, ascorbic acid derivatives, combination therapy, checkerboard test

Introduction

Propionibacterium acnes is an anaerobic, Gram-positive skin microbe that colonizes sebaceous glands and pilosebaceous follicles.[1] This organism is considered to play a principal role in the development of acne vulgaris.[1] Acne vulgaris is a chronic inflammatory disease characterized by typical inflammatory events, including the overproduction of sebum, abnormal desquamation of the sebaceous follicle epithelium, and *P. acnes* proliferation.[1–3] Regarding the pathogenic factors for acne development and aggravation, ultraviolet irradiation and peroxidation of sebum lipids have been reported to activate inflammatory mediators such as reactive oxygen species.[4]

Ascorbic acid derivatives are some of the most widely used antioxidants for protecting the skin.[5,6] The antioxidative effect of 5% sodium ascorbyl phosphate has demonstrated efficacy in acne vulgaris.[7] However, it remains unclear whether ascorbic acid derivatives have antimicrobial activity against *P. acnes*.

Many topical and systemic treatments have been proposed for acne vulgaris.[8] Clindamycin and erythromycin are the most frequently used agents against *P. acnes*.[8,9] On the other hand, many studies have reported the emergence of *P. acnes* with high

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1. This particular common bacteria is found around the sebaceous gland of the skin (the sebaceous gland is a product producing part of the body that opens into the hair that we see protruding from skin, it produces sebum, which is an oil that lubricates the hair). This common bacteria is known to induce acne in the face by production of reactive oxygen species (damaging oxygen atoms) leading to triggers of the gland to produce too much sebum by damaging the cells in that region. The outer layer of skin undergoes desquamation, where they peel off the rest of the skin (epidermis).cells.

2. P.Acne (the common bacteria in acne) has started to become resistant to many different antibiotics.

level resistance to macrolides including clindamycin because long-term antibiotic therapy is commonly used to treat acne vulgaris.[10–12] In Japan, a study conducted between 1994 and 1995 revealed that macrolide-resistant strains were found in only 4% (2/50) of *P. acnes* isolates from patients with acne vulgaris. However, they were found in 10% (5/48) of *P. acnes* isolates between 2006 and 2007, showing *P. acnes* resistance to macrolides has been increasing in Japan.[8,9]

Macrolide resistance in *P. acnes* is considered to be caused by mutation of the peptidyl transferase region in the domain V of 23S rRNA, and by the target site alteration with the 23S rRNA dimethylase that is encoded by *erm*(X).[13] In addition, these mutations is associated with cross resistance to clindamycin.[14] Therefore, the increasing prevalence of macrolide-resistant *P. acnes*, including clindamycin-resistant *P. acnes*, is a serious problem, because acne treatment by clindamycin is extremely difficult.

Recently, it has been reported that antibiotic combination enhances the therapeutic effect.[15,16] In this study, the in vitro efficacy of ascorbic acid derivates against *P. acnes* alone and in combination with clindamycin, erythromycin, chloramphenicol, minocycline, or levofloxacine was examined.

Material and methods

Bacterial strains and drugs

A total of 41 *P. acnes* strains used in this study were isolated from patients with acne vulgaris between 2006 and 2007 in Japan, and four strains of these were clindamycin-resistant *P. acnes* with mutation of the peptidyl transferase region in the domain V of 23S rRNA gene: A2058G, or A2059G.[9] The patients were given topical antibiotics instead of systemic antibiotics. The samples were cultured on modified Gifu anaerobic medium (GAM) agar (Nissui Pharmaceutical Co, Tokyo, Japan) under anaerobic conditions at 35°C for 72 hours. *P. acnes* was identified by Api 20A (bioMérieux, Marcy l'Etoile, France).[8] *P. acnes* JCM 6473 (ATCC 11828) strain was used as the quality control strain for antimicrobial susceptibility testing. Clindamycin, erythromycin, chloramphenicol, and minocycline were purchased from Sigma-Aldrich (Tokyo, Japan). All other chemicals used were of the highest analytical grade.

Susceptibility tests

Susceptibility testing was performed using microbroth dilution methods, according to the criteria of the Japanese Society of Chemotherapy.[17,18] The samples were cultured in GAM broth (Nissui Pharmaceutical Co) and adjusted to a McFarland standard number 1. A dilute bacterial suspension was used to inoculate wells of a 96-well microplate, each well containing a different concentration of the drug being tested. Double dilutions of the drugs were prepared. The concentrations of the drugs in the GAM broth ranged from 0.06 to 128 μg/mL (antimicrobial agents) or 1.25 to 1280 μg/mL (ascorbic acid derivatives), and a final concentration of 10^5 colony-forming units (CFU) of test bacteria per well was added to each dilution. The plates were incubated in an anaerobic gas-generating pouch (Anaero Pack System; Mitsubishi Gas Chemical Co, Tokyo, Japan) at 35°C for 48 hours. After the positive control lacking the antimicrobial agent demonstrated good growth, the minimum inhibitory concentration (MIC) for each antibiotic was defined as the lowest concentration of antibiotic required to inhibit bacterial growth, indicated by the absence of turbidity.

Fractional inhibitory concentration (FIC) index

The efficacy of a combination of zinc ascorbate and antimicrobial agents against *P. acnes* was determined by checkerboard tests using microbroth dilution methods.[19] Fractional inhibitory concentration (FIC) indices were calculated using the following formula: FIC index = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone).[20] An index of <0.5 was considered as synergism; that <1.0 but >0.5 as additive action; and that >2.0 as antagonism. The samples were adjusted to the McFarland standard number 1 and a final concentration of 10^5 CFU/well of test bacteria. The MICs of the combinations were determined after incubation at 35°C for 48 hours.

Results

The antibiotic susceptibility of *P. acnes* to ascorbic acid derivatives

The antibiotic susceptibility of *P. acnes* JCM 6473 to ascorbic acid derivatives zinc ascorbate, magnesium ascorbyl phosphate, and sodium ascorbyl phosphate was investigated (Table 1). The MIC of zinc ascorbate was 640 μg/mL, whereas the MICs of ascorbic acid, magnesium ascorbyl phosphate, and sodium ascorbyl phosphate were ≥1280 μg/mL. The normal dose of ascorbic acid derivatives is 5% (50 mg/mL).[7] These results indicate that zinc ascorbate sufficiently inhibits the growth of *P. acnes* in the normal dose. Thus, zinc ascorbate was used in further experiments.

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3. The researchers used 41 different strains of P.acnes (the bacteria) from patients with acne, as well as 4 strains of antibiotic resistant strains and cultured the bacteria to allow them to grow to enough of a concentration to use in experiments.

Table 1 Susceptibility of *Propionibacterium acnes* JCM 6473 to ascorbic acid derivatives

Ascorbic acid derivatives	MIC
Ascorbic acid	≥1280
Zinc ascorbate	640
Magnesium ascorbyl phosphate	≥1280
Sodium ascorbyl phosphate	≥1280

Abbreviation: MIC, minimum inhibitory concentration (μg/mL).

Combined effect of zinc ascorbate and various antimicrobial agents against *P. acnes*

To study the antimicrobial effects of combinations of zinc ascorbate and antimicrobial agents such as clindamycin, erythromycin, chloramphenicol, minocycline, and levofloxacin, the FIC index was determined by checkerboard tests (Table 2). In combinations of zinc ascorbate with clindamycin, erythromycin, or chloramphenicol, the MIC of zinc ascorbate reduced, and the FIC indices ranged from 0.625 to 0.75. The values exhibited an additive effect against *P. acnes* JCM 6473. Conversely, the FIC indices for the combinations of zinc ascorbate with minocycline or levofloxacin showed no effect.

Clindamycin is approved and commonly used in Japan for the treatment of acne vulgaris.[8,9] Thus, the antimicrobial effect of zinc ascorbate combined with clindamycin for 37 strains of clindamycin-sensitive *P. acnes* isolated from patients with acne vulgaris was investigated. As shown in Table 3, the MIC range of zinc ascorbate was approximately twofold lower in combination with clindamycin compared with that of zinc ascorbate and clindamycin alone. The average FIC index was 0.84, exhibiting an additive effect.

Table 2 Combined effects of zinc ascorbate and antimicrobial agents against *Propionibacterium acnes* JCM 6473

Drug	MIC (FIC index)	Interaction
Zinc ascorbate	640	
Clindamycin	0.03	
Erythromycin	0.13	
Chlorlamphenicol	2	
Minocycline	0.25	
Levofloxacin	0.5	
Zinc ascorbate/Clindamycin	80/0.02 (0.63)	Additive
Zinc ascorbate/Erythromycin	160/0.06 (0.75)	Additive
Zinc ascorbate/Chloramphenicol	160/1 (0.75)	Additive
Zinc ascorbate/Minocycline	640/0.25 (2)	Antagonism
Zinc ascorbate/Levofloxacin	640/0.5 (2)	Antagonism

Note: The interaction was defined as synergistic if the FIC index was <0.5, additive if the FIC index was between 0.5 and 1.0, antagonistic if the FIC index ≥2.
Abbreviations: MIC, minimum inhibitory concentration (μg/mL); FIC, fractional inhibitory concentration.

Combined effect of zinc ascorbate and various antimicrobial agents against clindamycin-resistant *P. acnes* isolated from patients with acne vulgaris

Clindamycin-resistant *P. acnes* with mutation of the peptidyl transferase region in the domain V of 23S rRNA has been increasing in Japan.[9] The antimicrobial effect of zinc ascorbate combined with clindamycin, erythromycin, and chloramphenicol for four strains (PA001–PA004) of clindamycin-resistant *P. acnes* with a transition of adenine to guanine at the position of 2058 (A2058G: PA001–PA003) or 2059 (A2059G: PA004) was investigated. As shown in Table 4, zinc ascorbate was equally effective at inhibiting the growth of clindamycin-resistant *P. acnes* strains (MIC 640 μg/mL). In combinations of zinc ascorbate with clindamycin, erythromycin, and chloramphenicol, the MIC of zinc ascorbate reduced (MIC range: 160–320 μg/mL). In addition, the FIC index of PA004 strain with clindamycin-resistant *P. acnes* was 0.75, exhibiting an additive effect.

Discussion

In the microbroth dilution methods, according to the criteria of the Japanese Society of Chemotherapy, a twofold serial dilution series is used to determine the effectiveness of the test drug, with 1 μg/mL as the primary dilution.[17,18] In this study, the concentrations of the drugs ranged from 0.06 to 128 μg/mL. However, ascorbic acid derivatives showed no effect in this concentration. It was confirmed that MIC of zinc was 1280 μg/mL (data not shown), which is similar to that reported by Fluhr et al.[21] Therefore, the concentrations of ascorbic acid derivatives ranged from 1.25 to 1280 μg/mL.

Five percent of sodium ascorbyl phosphate has been reported to reduce acne lesions by 20.14% and 48.82% within 4 and 8 weeks, respectively.[7] In addition, topical (1.2%) and systemic (30 mg) zinc have been reported to show efficacy in the treatment of acne vulgaris.[21,22] In the present study, the MIC (640 μg/mL) of zinc ascorbate against *P. acnes* JCM 6473 corresponded to 0.064%, and the MIC value was lower than other ascorbic acid derivatives (MIC ≥1280 μg/mL). Although further experiments are needed to examine the drug kinetics and the stability of zinc ascorbate, it is expected that the calculating concentration of zinc ascorbate would be 2800 μg/mL when it is applied on the skin (4 mg/cm^2) as an external preparation.[23] These results suggest a possibility that zinc ascorbate (MIC

Table 1

This table shows the tested concentrations of various ascorbic acid (Vitamin C) versions (derivatives) to see how much was needed to reach the "minimum inhibitory concentrations (MIC)" - the minimum amount of each ascorbic acid needed to noticeably inhibit bacterial growth.

Primary Results

- Zinc ascorbate needs half the amount to see the same amount of bacterial growth inhibition.

Take Away: Zinc ascorbate is the most potent ascorbic acid of the four tested.

Table 2

This table of data shows the exact same thing as Table 1, but including a variety of drugs used against acne by themselves, then again in combination with zinc ascorbic acid/ascorbate (Vitamin C).

Primary Results

- Some drugs paired with zinc ascorbic acid are more effective, others are not.

Take Away: Zinc ascorbic acid reduces the amount of anti-acne drug necessary to achieve reductions in P. Acne bacteria growth.

Table 3 Combined effect of zinc ascorbate and clindamycin against 37 strains of *Propionibacterium acnes* isolated from patients with acne vulgaris

Drug	MIC ranges (MIC_{90})		FIC		Interaction
	Alone	Combined	Range	Average	
Clindamycin	0.13–2 (2)	0.06–1 (1)	0.63–1	0.84	Additive
Zinc ascorbate	160–640 (640)	80–320 (160)			

Abbreviations: MIC, minimum inhibitory concentration (μg/mL); FIC, fractional inhibitory concentration; MIC_{90}, concentration of drugs which inhibited growth in 90% of the strains tested.

640 μg/mL) sufficiently inhibits the growth of *P. acnes* in the concentration that is lower than the normal dose of other ascorbic acid derivatives and zinc.

Choi et al[24] reported that the antimicrobial mechanism involves the capability of zinc ions to inhibit glycolysis of microorganisms by oxidizing thiol groups in essential glycolytic enzymes, therefore, the antimicrobial mechanism of zinc ascorbate may be similar to that of zinc. However, a combined effect of zinc ascorbate and minocycline or levofloxacin was not proved. Clindamycin, erythromycin, and chloramphenicol inhibit the protein synthesis of bacteria by binding to the 50S subunit of the bacterial ribosome.[25] In contrast, the binding sites of minocycline and levofloxacin are on the 30S subunit of the ribosome and on DNA topoisomerase IV, respectively.[26,27] Thus, the difference in antibacterial mechanism may be related to the combined effect of each of these. In addition, it is possible that minocycline and levofloxacin chelate with metal ions, including zinc,[28,29] resulting in reduced antimicrobial activity. Further experiments are needed to clarify the mechanism of zinc ascorbate against *P. acnes*.

Macrolides are used not only as antimicrobial agents but also as anti-inflammatory agents, and are the most commonly used to treat acne vulgaris.[9] Clindamycin shows a similar effect to macrolides and is the most popular topical antibacterial for acne vulgaris in Japan. However, clindamycin-resistant *P. acnes* strains have been increasing in Japan.[8,9] An increase of clindamycin-resistant *P. acnes* including macrolides and an epidemic of the multiple-drug-resistant *P. acnes* can be expected in the future. It is reported that combination therapy with antimicrobial agents and other agents such as the external retinoids and benzoyl peroxide is effective in preventing the emergence of the antibiotic-resistant strains of *P. acnes*.[15,16] In the present study, it was found that zinc ascorbate inhibited the growth of the clindamycin-resistant *P. acnes* strains (MIC 640 μg/mL). In addition, although the FIC of zinc ascorbate in combination with clindamycin was unable to be calculated because the MIC of clindamycin alone and in combination was ≥128 μg/mL (3/4 strains), it reduced the MIC of zinc ascorbate against all strains. Moreover, the FIC index of PA004 strain with clindamycin-resistant *P. acnes* was 0.75, exhibiting an additive effect. These results strongly suggest that the combination of zinc ascorbate and clindamycin will be useful for preventing the emergence of clindamycin-resistant *P. acnes* and for treating acne vulgaris.

Table 4 Combined effect of zinc ascorbate and antimicrobial agents against four strains of macrolides-resistant *Propionibacterium acnes* isolated from patients with acne vulgaris

Strain no.	MIC (FIC index)						
	AZn	CLDM	EM	CP	AZn/CLDM	AZn/EM	AZn/CP
PA001	640	≥128	≥128	2	160/≥128 (NC)	160/≥128 (NC)	320/1 (1)
PA002	640	≥128	≥128	2	320/≥128 (NC)	320/≥128 (NC)	320/1 (1)
PA003	640	≥128	≥128	1	320/≥128 (NC)	160/≥128 (NC)	160/0.5 (0.75)
PA004	640	64	≥128	1	160/32 (0.75)	320/≥128 (NC)	160/0.5 (0.75)

Abbreviations: MIC, minimum inhibitory concentration (μg/mL); FIC, fractional inhibitory concentration; PA, *Propionibacterium acnes*; AZn, zinc ascorbate; CLDM, clindamycin; EM, erythromycin; CP, chloramphenicol; NC, not calculated.

Table 3

The researchers are comparing the effectiveness of zinc ascorbate (Vitamin C) and an anti -acne drug (Clindamycin) either alone or in combination, but they are not just showing the minimum amount needed to begin seeing reductions in the acne bacteria, but also the concentration needed of each (in isolation or together) to see 90% reduction in bacteria (in parentheses).

Primary Result

- There is less needed, in combination, than in isolation.

Take Away: To reach 90% inhibition of bacteria, less of each (zinc ascorbate and clindamycin) is needed, implying they have an additive effect.

Table 4

The researchers are showing the minimum concentration needed to begin seeing reductions (minimum inhibitory concentration - MIC) of each drug with and without zinc ascorbate (Vitamin C) on the four strains of antibiotic resistant bacteria.

Primary Results:

- The drugs are presumed ineffectual at maximum concentration, except against strains PA004 for CLDM and CP drug.
- The same is true for zinc ascorbate.
- The drug concentrations are not reduced, except the CP condition, but the necessary zinc ascorbate concentration is reduced in need to elicit the same effect, when investigated in combination.

Take Away: Zinc ascorbate is effective at inhibiting antibiotic resistant bacteria.

Conclusion

The results of this study provide novel evidence that zinc ascorbate inhibits the growth of *P. acnes*, and its concentration (0.064%) is sufficiently lower than the normal dose (5%) of other ascorbic acid derivatives. In addition, zinc ascorbate shows an additive antimicrobial effect in vitro in combination with clindamycin, erythromycin, and chloramphenicol against clindamycin-sensitive and/or clindamycin-resistant *P. acnes*. These findings may provide novel insights into acne therapy. Recently, combination therapy with benzoyl peroxide and clindamycin has become common worldwide. Further experiments are needed to compare zinc ascorbate plus clindamycin and benzyol peroxide plus clindamycin.

Disclosure

The authors report no conflicts of interest in this work.

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