Resveratrol inhibition of *Propionibacterium acnes*

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Received 29 January 2007; returned 6 March 2007; revised 9 March 2007; accepted 12 March 2007

**Objectives:** To evaluate the effects of the anti-inflammatory hydroxystilbene, resveratrol, on *Propionibacterium acnes* growth.

**Methods:** Three different strains of *P. acnes* were tested against resveratrol at concentrations between 0 and 200 mg/L. Piceatannol was included as a second hydroxystilbene to compare with resveratrol, and erythromycin and benzoyl peroxide were used as positive controls.

**Results:** After 24 h of treatment with resveratrol, the average 50% inhibitory concentration (IC50) was 73 mg/L and the average 100% inhibitory concentration (IC100) was 187 mg/L for the three strains of *P. acnes* tested. The IC50 and IC100 of piceatannol were 123 and 234 mg/L, respectively. The highest concentration of resveratrol tested (200 mg/L) was bactericidal, whereas lower concentrations were bacteriostatic.

**Conclusions:** Resveratrol, an anti-inflammatory hydroxystilbene, is capable of inhibiting *P. acnes* growth.

Keywords: antimicrobial activity, drug development, human disease

**Introduction**

*Propionibacterium acnes* is a Gram-positive non-spore-forming anaerobic bacillus normally found on human skin and is intimately associated with acne vulgaris. Those areas of the body with the densest number of sebaceous glands are the most common sites of this disease and males are more frequently and severely affected than females. The lesions can be non-inflammatory, but more typically are inflammatory. Inflammation occurs when *P. acnes*, growing in plugged follicles, releases chemoattractants eliciting the inflammatory response creating the classical comedones of acne. Therefore, the clinical manifestations appear to be the result of bacterial-induced inflammation of a plugged sebaceous gland.

Because of the pathophysiology of the disease, an ideal treatment for such a condition would be a single drug capable of inhibiting *P. acnes* and also suppressing the inflammatory response. A candidate to fulfil these criteria is the hydroxystilbene, resveratrol (3,4,5-trihydroxystilbene). Resveratrol is a natural compound produced by some spermatophytes such as grapes and other plants. Resveratrol has been shown to be anti-inflammatory and also be active against several bacteria, including *Neisseria gonorrhoeae, Neisseria meningitidis*, *Helicobacter pylori*, *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *Proteus mirabilis*. Because of resveratrol’s reported inhibitory ability against some bacteria, we examined resveratrol for its effect on *P. acnes* in an attempt to identify an anti-inflammatory drug capable of suppressing *P. acnes* replication.

**Materials and methods**

Three strains of *P. acnes* (25746, 29399 and 33179) were obtained from the ATCC. The inhibitory concentration (IC) of the various drugs was determined by the broth dilution method according to CLSI (formerly NCCLS) M11-A6 guidelines. The IC50 was defined as that concentration of resveratrol that reduced bacterial growth, determined spectrophotometrically, by 50% and IC100 was defined as the lowest concentration of drug that inhibited bacterial growth by 100%. Endpoint readings for each drug concentration were done in triplicate with a microspectrophotometer at 600 nm. Because resveratrol is water-insoluble, it was dissolved in dimethyl sulfoxide (DMSO) and then added to actinomyces broth to a final concentration of 0–200 mg/L. Piceatannol (3,4,3',5'-tetrahydroxystilbene), which was included as a second...
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hydroxystilbene, was treated in an identical manner. The final concentration of DMSO was 0.2%. Benzoyl peroxide was dissolved in DMSO and added to its final concentration of 0–800 mg/L in broth. Its final concentration of DMSO was 1.6%. The final concentration of erythromycin in actinomyces broth was 0.01–10 mg/L. Erythromycin was dissolved in ethanol having a final concentration of 0.2%. DMSO and ethanol were included in the control cultures at concentrations equal to the highest concentration used to dissolve the drugs.

Twenty-four hour cultures of *P. acnes* were adjusted to 1 × 10⁶ cfu/mL using McFarland standards and inoculated into actinomyces broth lacking, or containing, a specific concentration of drug. Three tubes were inoculated for each drug concentration including controls lacking drug but containing DMSO or ethanol. Cultures were incubated anaerobically at 37°C and read spectrophotometrically at 24 and 48 h. A five-parameter sigmoidal curve was used to fit each data set and IC₅₀ and IC₁₀₀ calculated from these curves.

To determine whether resveratrol was bactericidal or bacteriostatic, CLSI M26-A guidelines⁸ were followed for all three strains of *P. acnes*. Briefly, 24-h-old *P. acnes* cultures were adjusted to 5 × 10⁵ cfu/mL in actinomyces broth with or without a specific concentration of resveratrol (0–200 mg/L). The cultures were incubated anaerobically at 37°C for 12 or 24 h and then plated on agar plates. After 48 h of anaerobic incubation at 37°C, colonies were counted.

Growth rates of all strains of *P. acnes* were obtained by starting with 1 × 10⁶ cfu/mL. The cultures were incubated for 4, 8, 12 or 16 h under anaerobic conditions at 37°C and then plated on agar and incubated again for an additional 48 h before colonies were counted. The average bacterial concentrations were plotted against time. The generation time was determined by comparing the concentration of bacteria at the beginning of the study with the bacterial concentrations at later time points. The standard equation for geometric growth was used to quantify cell division. The equation used was

\[
\frac{n}{t} = \frac{3.3 \log \text{number of bacteria at the end of the time interval}}{\text{time interval}}
\]

number of generations. The number of generations was calculated by

\[n = 3.3 \log \text{number of bacteria at the end of the time interval} \]

Results

All drugs inhibited the replication of all strains of *P. acnes* (Table 1). After 24 h of erythromycin treatment, the average IC₅₀ for all strains of *P. acnes* was 1.5 mg/L and the IC₁₀₀ was 11 mg/L. The average IC₅₀ for resveratrol was 73 mg/L and the average IC₁₀₀ was 187 mg/L. The IC₅₀ and IC₁₀₀ of piceatannol were 123 and 234 mg/L, respectively. Benzoyl peroxide treatment had an average IC₅₀ of 164 mg/L and an average IC₁₀₀ of 295 mg/L. Similar results were obtained with all the drugs after 48 h of treatment (Table 1).

Studies were undertaken to determine whether resveratrol was bactericidal or bacteriostatic. Overall, the number of bacterial colonies formed decreased for all strains as the concentration of resveratrol increased (Table 2). The highest concentration of resveratrol tested (200 mg/L) exhibited bactericidal activity by completely inhibiting bacterial growth of all strains of *P. acnes* after 12 and 24 h of treatment (Table 2). The other two concentrations (50 and 100 mg/L) of resveratrol were bacteriostatic, reducing colony formation of all strains after 12 h of treatment with the drug. However, after 24 h of treatment with either 50 or 100 mg/L resveratrol, no colonies were formed with ATCC strain 25746, whereas the other two strains produced fewer colonies when compared with non-treated controls.

As each strain reacted differently to the various concentrations of resveratrol, we investigated their generation time. ATCC strain 25746 had the fastest replication time of 3.3 h and was the most susceptible to treatment with resveratrol at 50 mg/L. ATCC strain 29399 had the slowest generation time of 5.1 h and was the least responsive to resveratrol treatment at 50 mg/L. ATCC strain 33179 had an intermediate generation time of 4.8 h and was moderately inhibited by treatment with 50 mg/L resveratrol.

Discussion

In this study, we examined the effects of the anti-inflammatory drug resveratrol on three strains of *P. acnes*. The results showed that resveratrol inhibited *P. acnes* and was bactericidal at the highest concentration tested. The results also demonstrated that the bacterium with the fastest generation time was more susceptible to the inhibitory effects of resveratrol than those with slower generation times. When compared with commonly used treatments of acne vulgaris, erythromycin and benzoyl peroxide, resveratrol compared favourably.

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<th>Table 1. Inhibitory concentrations of various drug treatments for three different strains of <em>Propionibacterium acnes</em></th>
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Acne vulgaris is the result of the combined action of *P. acnes* infection and the inflammatory response to that infection. Resveratrol presents a potentially interesting treatment because our results show that it inhibits *P. acnes* replication and others have shown that it has anti-inflammatory properties. Because of the ability of resveratrol to inhibit *P. acnes*, its anti-inflammatory properties and its ready availability, resveratrol may be used to benefit those suffering from *P. acnes*-associated disease.

### Acknowledgements

Funding for this study was provided by a grant from Royalmount Pharma, Inc., Montreal, Quebec, Canada.

### References


