

Evaluation of Anti-Bacterial Drugs Using Silkworms Infected by *Cutibacterium Acnes*

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Research note

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Abstract

Objective: *Cutibacterium acnes* is a causative agent of inflammatory skin diseases and systemic infections. Systemic infections caused by *C. acnes* are difficult to treat, and the development of a systemic infection model for *C. acnes* would be useful for elucidating the mechanisms of infection and searching for therapeutic agents. In this study, we established a silkworm infection model as a new experimental system to evaluate the interaction between *C. acnes* and the host, and the efficacy of antibacterial drugs.

Results: Silkworms infected with *C. acnes* died when reared at 37°C. The dose of injected bacterial cells required to kill half of the silkworms (LD₅₀) was determined under rearing conditions at 37°C. Silkworms injected with autoclaved *C. acnes* cells did not die during the study period. The survival time of silkworms injected with *C. acnes* was prolonged by the injection of antibacterial drugs such as tetracycline and clindamycin. These findings suggest that the silkworm *C. acnes* infection model can be used to evaluate host toxicity caused by *C. acnes* and the *in vivo* efficacy of antimicrobial drugs.

Introduction

Cutibacterium acnes (formerly *Propionibacterium acnes*), a common bacterium on human skin, causes inflammatory skin diseases and systemic infections (1, 2). *C. acnes* is isolated as the predominant species in 34% (3) or 36.2% (4) of intervertebral discs removed from patients with chronic low back pain, such as disc herniation. Biofilm formation by *C. acnes* on implants and on intervertebral discs causes bloodstream infections (5–7). Because *C. acnes* forms a biofilm and more than 50% of clinically isolated *C. acnes* is resistant to typical macrolides, systemic infections caused by *C. acnes* are difficult to treat (8, 9). Therefore, the development of treatments for systemic infections caused by *C. acnes* is clinically important. Although mammalian animal models of *C. acnes* infection have been established, their use for the evaluation of antibacterial drugs is difficult due to the long duration of the infection (10, 11). Infection experiments using a large number of mammalian animals are also difficult to perform due to animal welfare issues (12).

Silkworms are useful animals for assessing host-pathogen interactions in systemic infections and for evaluating the therapeutic effects of antimicrobial drugs (12, 13). Because silkworms also have advantageous features such as easy rearing in large numbers in a small space with few ethical issues, experiments with large numbers of silkworms can be performed (14). Moreover, quantitative drug administration and monitoring of parameters in silkworm blood can be performed due to ease of sample injection and blood collection (12, 15, 16). The use of a silkworm infection model based on these advantageous features led to the discovery of virulence-related genes for pathogenic microorganisms such as *Staphylococcus aureus*, *Candida albicans*, and *Candida glabrata* (17–19). Further, exploratory studies of antimicrobial drugs using a silkworm infection model led to the identification of compounds exhibiting therapeutic efficacy in mouse infection experiments (20–22). Therefore, the silkworm infection

model is useful for studies aimed at elucidating the infection mechanisms of pathogenic microorganisms and evaluating the efficacy of antimicrobial drugs.

In the present study, we attempted to establish an animal model of systemic infection by *C. acnes* using silkworms. We found that injection of *C. acnes* cells killed silkworms. Survival times of the infected silkworms were prolonged by injection of tetracycline and clindamycin. Our findings suggest that the silkworm infection model with *C. acnes* is useful for evaluating the efficacy of antimicrobial drugs.

Materials And Methods

Reagents

Tetracycline was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Clindamycin was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). These reagents were dissolved in physiologic saline solution (0.9% NaCl). GAM agar was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan).

Culture of *C. acnes*

C. acnes ATCC6919 strain was used in this study. The *C. acnes* ATCC6919 strain was grown on GAM agar plates at 37°C under anaerobic conditions.

Silkworm infection experiments

The silkworm infection experiments were performed as previously described (23). Silkworm fifth instar larvae were fed an artificial diet (1.5 g; Silkmate 2S; Ehime-Sanshu Co., Ltd., Ehime, Japan) overnight. *C. acnes* grown on GAM agar plates was suspended in physiologic saline (0.9% NaCl). A suspension (50 µl) of the *C. acnes* cells was injected into the silkworm hemolymph with a 1-ml tuberculin syringe (Terumo Medical Corporation, Tokyo, Japan). Silkworms injected with the *C. acnes* cells were placed in an incubator and survival was monitored.

Statistical analysis

The significance of differences between groups was calculated using a log-rank test based on the Kaplan-Meier method using Prism 9 (GraphPad Software, LLC, San Diego, CA, USA, <https://www.graphpad.com/scientific-software/prism/>). $P < 0.05$ was considered a statistically significant difference.

Results

Pathogenicity of *C. acnes* against silkworms

Silkworm models of infection by various microorganisms have been established (14). The body temperature of silkworms, which can be regulated by changing the rearing temperature, is important for

bacterial pathogenicity against silkworms (14). *C. acnes* grows at approximately 32°C on the skin and 37°C in the human body. We therefore examined the rearing temperatures that caused death in *C. acnes*-infected silkworms. Silkworms injected with *C. acnes* (1.6×10^9 cells) reared at 37°C after injection died within 40 h, whereas infected silkworms reared at 32 °C survived longer (Fig. 1a and b). The time required for half of the infected silkworms to die (LT₅₀) was 48 h for silkworms reared at 32°C and 27 h for those reared at 37°C (Fig. 1a and b). The LD₅₀ value, which is the bacterial number required to kill half of the silkworms, was approximately 2×10^8 cells when infected silkworms were reared at 37°C (Fig. 1c). These results suggest that rearing *C. acnes*-infected silkworms at 37°C decreased survival and that the pathogenicity of *C. acnes* can be quantitatively assessed based on the LD₅₀ value.

Effect of heat-killed *C. acnes* cells on silkworms

We next examined the heat-killed cells to evaluate whether *C. acnes* cells must be alive to exert pathogenicity against silkworms. Injection of *C. acnes* cells killed silkworms, but injection of autoclaved *C. acnes* cells did not (Fig. 2). These results suggest that the pathogenicity of *C. acnes* in silkworms depends on the viability of the *C. acnes* cells.

Therapeutic effects of antibacterial drugs against silkworms infected with *C. acnes*

We next examined the efficacy of antibacterial drugs in the silkworm *C. acnes* infection model. Administration of tetracycline and clindamycin to silkworms infected with *C. acnes* prolonged the survival time (Fig. 3). These results suggest that the efficacy of antibacterial drugs can be evaluated using the silkworm *C. acnes* infection model.

Discussion

In this study, we demonstrated that *C. acnes* kills silkworms reared at 37°C and that the silkworm infection model can be used to evaluate the efficacy of antibacterial drugs. Our findings suggest that the silkworm infection model is useful for assessing pathogenicity and the efficacy of antimicrobial drugs against systemic infection by *C. acnes*.

Bacterial components such as peptidoglycans of *Porphyromonas gingivalis* lead to shock in silkworms, resulting in their death (24). Injection of viable or heat-killed *P. gingivalis* cells cause silkworm death (24). Under conditions in which shock occurs, silkworms cannot be treated with antibiotics (24). In the silkworm *C. acnes* infection model established in this study, administration of heat-killed bacteria did not kill the silkworm. Further, *C. acnes*-infected silkworms could be effectively treated with antibacterial drugs, suggesting that the growth of *C. acnes* in the body of silkworms is important for its pathogenicity. Silkworm infection models are useful for identifying virulence factors of pathogenic microorganisms (13) (14). Further studies are needed to determine which factors in *C. acnes* are responsible for the pathogenicity to silkworms.

The pharmacokinetics of antimicrobial agents are similar between silkworms and mammals, and antimicrobial drug efficacy in the silkworm infection model can be evaluated on the basis of the pharmacokinetics (21, 25–27). Therefore, silkworm infection models are useful for the development for *in vivo* screening of new antimicrobial agents (20, 22). The silkworm *C. acnes* infection model with may be useful for identifying effective antibacterial compounds against systemic infection by *C. acnes*. Azole antifungals exhibit antimicrobial activity against *C. acnes in vitro*, and ketoconazole inhibits the lipase activity of *C. acnes* (28, 29). Further studies are needed to identify effective compounds against systemic *C. acnes* infections from among clinically applied drugs using the silkworm infection model.

Recently, an infection model with *C. acnes* using a nematode, *Caenorhabditis elegans*, was reported (30). *C. elegans* is useful for identifying host factors against *C. acnes* infection based on genetic approaches (30). The differences between the silkworm system and the *C. elegans* system are that the silkworm blood can be directly injected with *C. acnes* and its pathogenicity at 37°C, the same temperature as the human body, can be verified. *C. elegans* is difficult to inject quantitatively into body fluids and cannot grow at 37°C (31). The silkworm infection model might allow us to identify virulence factors of *C. acnes* at the body temperature of humans.

In conclusion, we established a silkworm infection model with *C. acnes* and found the system to be useful for evaluating antibacterial drug efficacy. Further studies are needed to determine the clinical applicability of research using the silkworm *C. acnes* infection model.

Limitations

The results of this study are limited to one strain and only a few antibacterial drugs. Further *in vivo* studies for chemical screening are required to clarify whether the silkworm infection model could be effective for identifying candidate compounds.

Abbreviations

Saline: physiologic saline solution.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All datasets generated during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YM designed the study, performed examinations, and wrote the initial draft of the manuscript. YT contributed to data collection. TS contributed to data interpretation and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

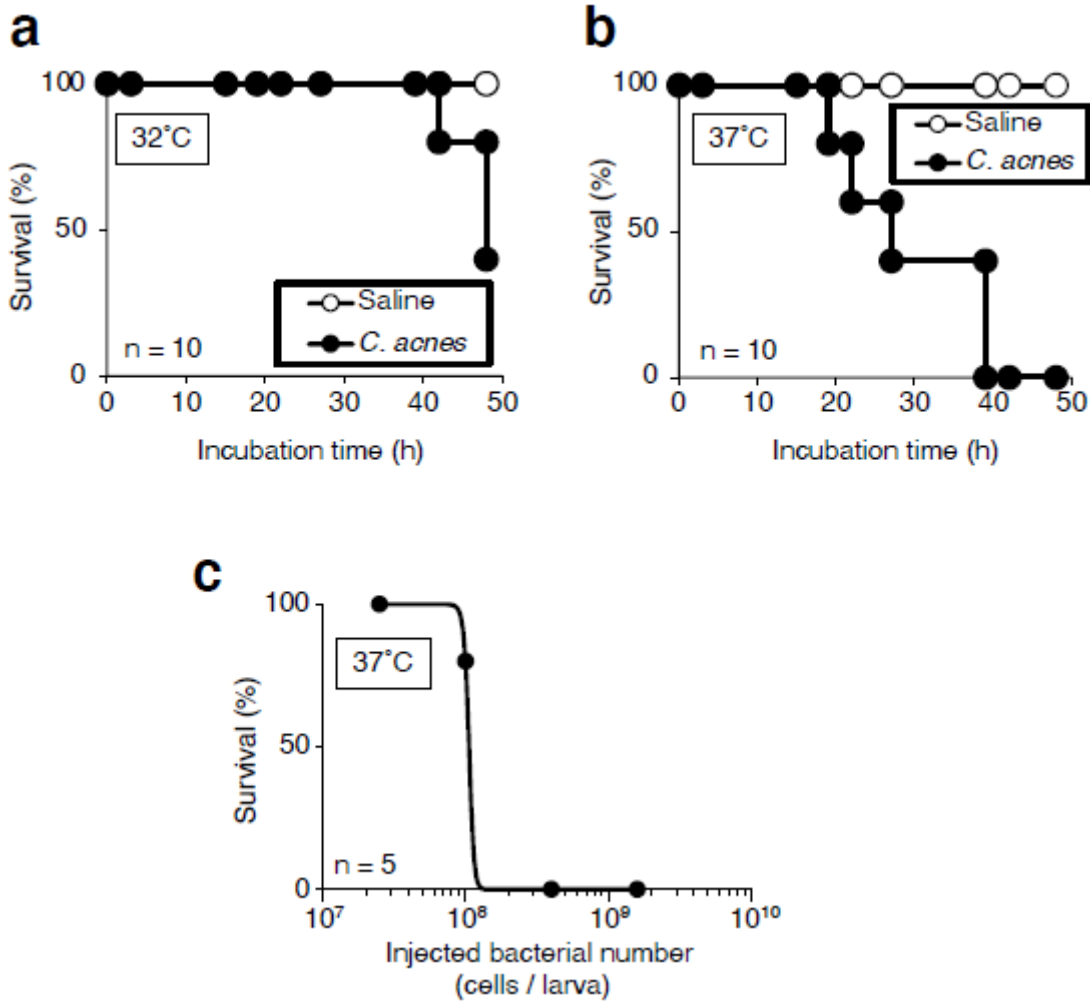


Figure 1

Toxic effects of *C. acnes* injected into silkworms. Silkworms were injected with saline or *C. acnes* cell suspension (1.6×10^9 cells) and incubated at 32°C (a) and 37°C (b). Number of surviving silkworms was measured over time. $n = 10$ /group. c Silkworms were injected with saline or *C. acnes* cell suspension ($2.5 \times 10^7 - 1.6 \times 10^9$ cells) and incubated at 37°C. Number of surviving silkworms was measured at 48 h after injection. $n = 5$ / group.

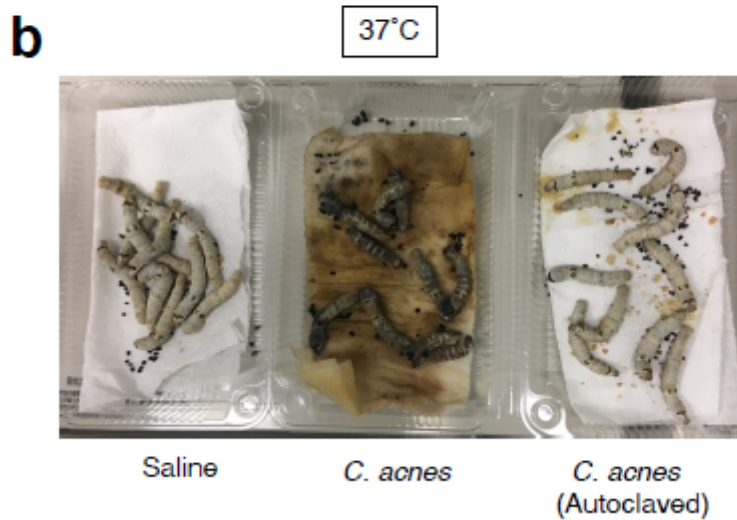
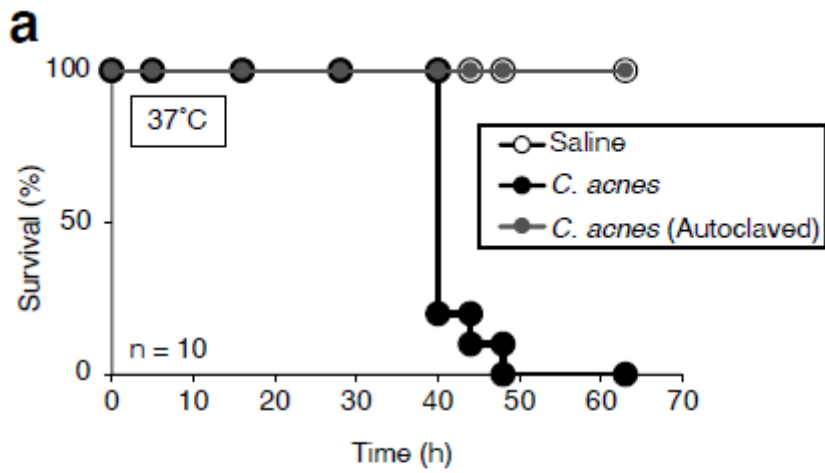


Figure 2

Effects of injecting autoclaved *C. acnes* cells in silkworms a Silkworms were injected with saline, *C. acnes* cell suspension (7.8×10^8 cells), or autoclaved *C. acnes* cell suspension and incubated at 37°C. Number of surviving silkworms was measured over time. n = 10 / group. b Picture at 48 h after injection is shown.

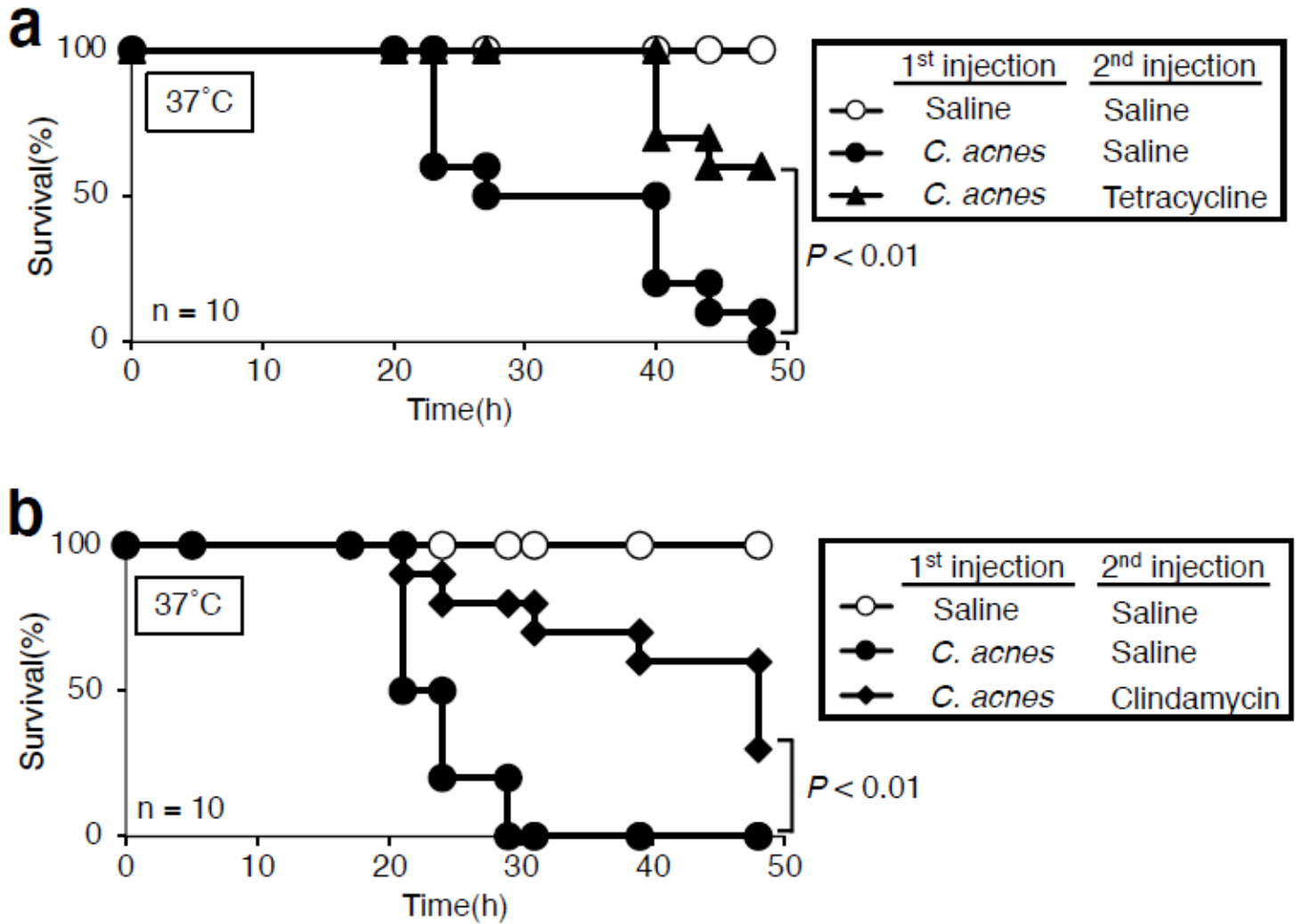


Figure 3

Effect of antibacterial drugs in silkworms infected by *C. acnes* Silkworms were injected with saline or *C. acnes* cell suspension (4.9×10^8 cells (a) or 1.6×10^9 cells (b)), and then with tetracycline ($100 \mu\text{g} / \text{larva}$) (a) or clindamycin ($100 \mu\text{g} / \text{larva}$) (b). Number of surviving silkworms under incubation at 37°C was measured over time. Statistically significant differences between groups were evaluated using a log-rank test. $n = 10 / \text{group}$.