# Representative species, skin diseases and Kampo medicines

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**We examined the relationship between the *Propionibacterium* species and Kampo formulations, between the *Staphylococcus* species and Shiunko, or between acne vulgaris and Kampo formulations. *Jumihaidokuto, Unseiin* and Shiunko suppressed *Propionibacterium* species lipase, and in correlation with the lipase activity, their clinical effect was indicated. Among Shiunko, white petrolatum and 3.5% salt water, Shiunko strongly inhibited the growth of the *Staphylococcus* species isolated from skin lesions of atopic dermatitis. Also, Kampo formulations were effective for inflammatory acne rash as well as incidental symptoms in acne patients. Especially, the effects of Kampo formulations on the incidental symptoms were remarkably higher than those of antimicrobial agents. These effects might be due to the various actions of Kampo crude drugs composed of Kampo formulations or Shiunko.**

**Key words** *Propionibacterium* species, *Staphylococcus* species, Kampo formulation, Shiunko, acne vulgaris, atopic dermatitis, incidental symptoms.

## Introduction

In the Japanese dermatological field, Kampo formulations and skin diseases are sometimes discussed, and the use of Kampo formulations to treat skin diseases has increased steadily since they were approved by the Japanese Healthcare System in 1976. Instead, the discussion referred to representative organisms (aerobes and anaerobes) in the dermatological field and Kampo formulations have hardly performed well.

In this period, we evaluated the representative species, such as *Propionibacterium* and *Staphylococcus* species, and some Kampo medicines, or skin diseases and Kampo formulations.

## *Propionibacterium acnes* lipase, Jumihaidokuto and Shiunko

Acne vulgaris is not a simple common disease, which may often lead to psychosomatic influence and *P. acnes* lipase is the important pathogenic factor in acne vulgaris. Kampo formulations are usually composed of many natural substances, such as medical plants, which might have clinical effects. Kampo formulations are noted traditional medical prescriptions that have seen use in treating acne vulgaris or other skin diseases, either alone or in conjunction with Western therapy. Jumihaidokuto (JHT) was the representative Kampo formulation frequently used in the dermatological field. The target group of JHT was acne vulgaris or mild exudation transition to purulent dermatitis.[1] Many Kampo crude drugs composed of JHT have anti-bacterial and inflammatory activities. Through their activities, JHT significantly inhibited *P. acnes* growth and secondary decrease *P. acnes* lipase activity. Quantitative analysis of lipase was carried out by our method as follows.[2] Tested strains were incubated at 37℃ for 48 hours in Peptone-Yeast-Glucose medium supplemented with tributyrin and JHT. NaCl, diethyl ether was added and centrifuged at 3,000 rpm at 4℃ for 15 min and 2 μl of sample was injected into the gas chromatograph, and the lipase activity was calculated from the area of butyric acid on the gas chromatograms.

We also found that detachment of the cell wall, and the cytoplasmic degeneration of *P. acnes* grown in medium containing sub-MIC of JHT was observed under electron microscopy and this finding suggested damage of *P. acnes* lipase production.[3] The inhibition of *P. acnes* lipase activity by JHT was considered to be due chiefly to its main ingredients, *Glycyrrhizae radix* (GR) and *Platycodi radix* (PR). Flavonoids are important ingredients of GR, which have a strong anti-organismic activity, particularly against Gram-positive bacteria, and thus lead to the suppression of *P. acnes* lipase. It is well known that saponin, which is an ingredient of PR, also has an antibacterial activity.

Shiunko, a Kampo ointment, composed of several extracts (*Oleum sesami, Lithospermi radix, Angelicae radix* (AR), *Cera alba* and *Adeps suillus*), has many activities including an antimicrobial activity, and has been more recently used for acne vulgaris. A similar tendency to inhibit *P. acnes* lipase elicited by JHT was also seen in Shiunko. Shiunko (0.01% and 0.1%) suppressed *P. acnes* lipase activity about 8 and 14%, respectively.[4] If the clinical effects of JHT or Shiunko differ at the various acne sites, the inhibition of *P. acnes* lipase activity might also differ at those sites. Among the Kampo crude drugs, obvious differences in *P. acnes* lipase inhibition were observed. The synergistic actions have already been observed between Kampo formulations, Kampo crude drugs or its ingredients.[5] The clinical effects of Kampo formulations to acne vulgaris might be due to their antioxidant actions on infiltrating neutrophils as well as on their inhibition of *P. acnes* lipase activity was already improved.[6]

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**Table 1** Numbers of *Staphylococcus* species isolated from the skin of patients with atopic dermatitis before and after treatment with Shiunko, white petrolatum or salt water

| | Before/after treatment with | | |
|---|---|---|---|
| | Shiunko | White petrolatum | Salt water |
| | Isolates | Isolates | Isolates |
| 1 | S. aureus (+)/<br>S. aureus (+) | Not detected/<br>Not detected | Not detected/<br>S. aureus (++) |
| 2 | S. aureus (++)/<br>S. aureus (+) | S. warneri (+)/<br>S. warneri (+) | S. aureus (+)/<br>S. aureus (+) |
| 3 | S. aureus (++)/<br>S. aureus (+) | S. aureus (+)<br>S. warneri (+)/<br>S. aureus (+) | S. aureus (+)<br>S. warneri (+)/<br>Not detected |
| 4 | Not detected/<br>Not detected | S. aureus (+)/<br>Not detected | S. aureus (+)<br>S. epidermidis (+)/<br>S. aureus (++) |
| 5 | S. aureus (+)<br>S. epidermidis (++)/<br>S. aureus (+) | S. aureus (+)/<br>S. aureus (+++) | S. aureus (+)/<br>S. aureus (+++) |
| 6 | S. aureus (++)/<br>S. aureus (+) | S. epidermidis (+)/<br>S. haemolyticus (++) | S. haemolyticus (+)<br>S. capitis (+)/<br>S. aureus (++) |
| 7 | S. aureus (+++)/<br>S. aureus (++) | S. haemolyticus (++)/<br>S. aureus (++)<br>S. warneri (+) | S. aureus (+++)/<br>S. aureus (+++)<br>S. haemolyticus (+++) |

Number of bacteria:+++, high (over 500) ;++, moderate (51 to 500) ;+, low (1 to 50 CFU/cm$^2$)

Shiunko has often been used for burns and hemorrhoids, and more recently for atopic dermatitis.[7] We studied the bacterial numbers on 7 atopic dermatitis patients before and after 3 weeks topical application of Shiunko, white petrolatum or 3.5% salt water.[8] Reduced *S. aureus* counts were seen in 5 patients treated with Shiunko, one treated with white petrolatum and one with 3.5% salt water (Table 1). Treatment with 3.5% salt water might be less effective than Shiunko, because *S. aureus* is salt tolerant and can grow on medium containing 7% salt. White petrolatum might be also less effective than Shiunko since it is a protective material with little bactericidal action. Higher concentration of 3.5% salt water might induce side effects such as skin irritation. Shiunko is an economical ointment which does not induce undesirable severe skin side effects; it may be proved effective against many skin infections whose main causative agents are *S. aureus*, such as ulcer or secondary infections. But, long term therapy of Shiunko must be followed to avoid the appearance of bacteria with multiple drug resistance.

## *Propionibacterium avidum* and Unseiin

Among *Propionibacterium* species, *P. acnes* as well as *Propionibacterium granulosum* are well known for the cause of acne vulgaris or sarcoidosis. Free fatty acid produced by *Propionibacterium avidum* lipase is known as the cause of a bad smell in axillary osmidrosis. The infections induced by *P. avidum* have been reported,[9] nevertheless the studies referred to *P. avidum* have been hardly performed.

*Unseiin* (UI), which is the representative Kampo formulation, has been used for eczema, psoriasis, disorders of sweat glands, menopause-syndrome, and others.[10] Yasue mentioned[11] that UI was indicated for pruritus cutaneous universalis, especially when the desquamation, dry skin, skin atrophy and eczematous change accompanied it.

We examined *P. avidum* lipase produced from axillary osmidrosis and axillary normal skin and UI as its inhibitor.[12] Four *P. avidum* from axillary normal skin and 4 *P. avidum* from axillary osmidrosis were used. Butyric acid production was higher in axillary osmidrosis (2.77 ± 0.33 meq/ml) than in axillary normal skin (1.94 ± 0.17 meq/ml) (significant, paired t-test, p=0.047). The acid production was suppressed by 1mg/ml UI in axillary osmidrosis and axillary normal skin. A significant statistical result was not obtained, but more suppression of *P. avidum* lipase was seen in axillary osmidrosis (Table 2). Among *Propionibacterium* species isolated human skin, the predominant species were *P. acnes*, next *P. granulosum* or *P. avidum*. *P. avidum* was well isolated from moist areas, such as nose or axilla.

**Table 2** Production of butyric acid from *Propionibacterium avidum*

| | Control (meq/ml) | 1mg/ml Unsei-in (meq/ml) (reduction) |
|---|---|---|
| Normal skin | | |
| *P. avidum* TM100 | 1.56 | 1.23 (23%) |
| TM101 | 1.86 | 1.56 (16%) |
| TM102 | 2.20 | 1.86 (16%) |
| TM103 | 2.12 | 1.68 (26%) |
| Average±standard error | 1.94±0.17 | 1.58±0.15 (20%) |
| Osmidrosis | | |
| | $p$=0.047 | Not significant |
| *P. avidum* TM110 | 2.82 | 2.30 (18%) |
| TM111 | 3.20 | 2.36 (26%) |
| TM112 | 3.10 | 2.56 (18%) |
| TM113 | 1.96 | 1.32 (33%) |
| Average±standard error | 2.77±0.33 | 2.14±0.32 (24%) |

In these areas, the density of *P. avidum* was often the same as that of *P. acnes*.[13] It was widely known that *P. avidum* was a weak pathogenic organism, but high lipase activity of *P. avidum* might lead to more activation of apocrine sweat gland and a bad smell. *P. avidum* did not require much skin lipid for nutrition, but the correlation with *P. avidum* and triglycerides in skin lipids or interactions between microorganisms in the axilla are as yet unknown.

Of course, enzyme of species other than *P. avidum* should not be neglected.

KRT composing UI were *Rehmanniae Radix, Paeoniae Radix (PaR), Cnidii Rhizoma (CnR), AR, Scutellariae Radix (SR), Phellodendri Cortex (PC), Coptidis Rhizoma (CR)* and *Gardeniae Fructus*. *PaR, SR, PC* and *CR* had antibacterial activities. *PaR*, which mainly consisted of paeoniflorin and oxypaeoni-florin, had the activity to Gram-positive bacteria. *SR*, which mainly consisted of flavonoid, had the activity to *Propionibacterium* species. *PC* and *CR*, which mainly consisted of alkaloid, also had the activities to Gram-positive bacteria. In addition, *PaR, SR, PC, CR* and *AR* (chief components: ligustilide and n-butylidenphthalide) had anti-inflammatory activities. These activities were indicated to be closely connected with anti-lipase activity. UI suppressed well *P. avidum* lipase produced from axillary osmidrosis and axillary normal skin. Probably, UI might suppress other *Propionibacterium* species lipase. These activities might lead to the clinical effect of moist lesion and bad smell of axillary osmidrosis. It was likely that some concentration of UI in skin tissue existed, but the method to measure the concentration had not been established.

We have sometimes missed the importance of Kampo formulations having antibacterial effects and bacteria. Recently, defense of infection by Kampo formulations, especially a complementary drug, has been emphasized. Indeed, the strong effect of Kampo formulations against *Staphylococcus aureus* or other organisms have been reported.[14] The significance of *P. avidum* has been reported.[8] We should pay attention to *P. avidum* and to use UI, especially when a high dose of *P. avidum* is isolated or when only *P. avidum* was repeatedly isolated from the same infectious site. We should evaluate the possibility of UI application for prophylaxis or other skin diseases.

## Acne vulgaris, Seijobofuto, Jumihaidokuto and Tokishakuyakusan

Many Kampo formulations have been used for acne therapy and many studies which referred of their suppression of acne rashes have been reported.[15] Conversely, suppression of Kampo formulations to incidental symptoms of acne vulgaris has hardly been studied. We discussed the suppression of *Seijobofuto* (SBT), *Jumihaidokuto* (JHT) and *Tokishakuyakusan* (TSS) to the rashes as well as incidental symptoms of acne patients, and further compared them to the case of antimicrobial agents which have been frequently used for acne therapy.[16] We followed 71 acne patients. SBT, JHT and TSS, were used for the 17, 18 and 11 patients during 3 months, respectively. One antimicrobial agent was used for the remaining 25 patients during 3 months. Before and after use of these agents, we investigated the degree of red papule and pustule, and the existence of incidental symptoms of acne vulgaris. The degree of rash after using the agents compared to before was indicated as effective, slight effective and no change, respectively. The 5 incidental symptoms used were red cheeks, dysmenorrhea, constipation, cold hands/feet and pale face. Acne patients themselves judged the existence of these incidental symptoms.

The effects of SBT, JHT and TSS on rashes were similar to those of antimicrobial agents. Those on the 5 incidental symptoms were generally higher than those of antimicrobial agents. Effects were lower on symptoms of pale faced patients treated with SBT and on constipation in patients treated with JHT (Table 3).

Some Kampo crude drugs composed of SBT have antimicrobial activities. *Glycyrrhizae Radix* (GR), *Platycodi*

**Table 3** Effects of inflammatory acne rash and incidental symptoms in acne patients by Kampo formulations and antimicrobial agents

| Rash/Incidental Symptoms | Effect | Seijobofuto (n=17*) | Jumihaidokuto (n=18) | Tokishakuyakusan (n=11) | Antimicrobials (n=25) |
|---|---|---|---|---|---|
| Red papules | No change and worsening | 12% | 6% | 9% | 12% |
| | Slightly effective | 24% | 38% | 27% | 28% |
| | Effective | 64% (88%**) | 56% (94%) | 64% (91%) | 60% (88%) |
| Pustules | No change and worsening | 12% | 6% | 18% | 8% |
| | Slightly effective | 29% | 22% | 9% | 28% |
| | Effective | 59% (88%) | 72% (94%) | 73% (82%) | 64% (92%) |
| Red cheeks | | 100% (9[a]/9[b]) | 67% (6/9) | 44% (4/9) | 33% (4/12) |
| Dysmenorrhea | | 56% (5/9) | 60% (6/10) | 75% (6/8) | 0% (0/7) |
| Constipation | | 33% (3/9) | 20% (2/10) | 67% (6/9) | 15% (2/13) |
| Cold hands/feet | | 70% (7/10) | 50% (5/10) | 44% (4/9) | 0% (0/15) |
| Pale face | | 29% (2/7) | 50% (3/6) | 80% (8/10) | 11% (1/9) |

* Number of patients;
** Slightly effective + effective
[a] Number of patients with improvement of each incidental symptoms
[b] Number of patients with each incidental symptom

*Radix* (PR), *Scutellariae Radix* (SR), *Coptidis Rhizoma* (CR), *Schizonepetae Spica* (SS) and *Aurantii Fructus Immaturus* have anti-inflammatory activities,[17] and thus these accelerated effects might lead to improvement in rashes. PR, SR, CR and SS affect blood pressure and temperature,[18] and these accelerated effects might also lead to red cheeks and/or to cold hands and feet. Apparently, the 2 incidental symptoms were inverted but the effect found might have act as an adjustment. The rate of constipation by SBT was lower than that induced by TSS, because TSS provokes strong peristalis of the intestinal tract.

In acne vulgaris, JHT has been considered effective for pustules. JHT was also more effective in reducing pustules than SBT or TSS. Among Kampo crude drugs composing JHT, GR and PR have antimicrobial and anti-inflammatory activities, and, *Ledebouriella Radix* (LR), *Hoelen,* SS and *Bupleuri Radix* (BR) have only anti-inflammatory activities.[19] This might lead to an effect on the rashes. The effect against red cheeks, pale face and cold hands/feet might be due to the action of, PR, LR, *Hoelen,* SS and BR on blood pressure/temperature action.[20] The effect on dysmenorrhea might also be done to the sex hormone activity of, GR and *Hoelen.*[21] The indication of JHT should be chiefly the existence of red cheeks, dysmenorrhea and pustule.

TSS was effective in treating dysmenorrhea, pale face and constipation. TSS is composed of, *Atractylodis Lanceae Rhizoma* (ALR), PaR, *Hoelen,* AR, CnR and *Alismatis Rhizoma.* PaR has antimicrobial activity, and AR and *Hoelen* have anti-inflammatory activity. ALR also has both activities. PaR and Hoelen have sex hormone activity and actions on blood pressure/temperature,[22] both lead to an effect of a pale face. TSS was effective in treating rashes, although the kinds of Kampo crude drugs with antimicrobial and/or anti-inflammatory activities were lower. Antimicrobial agents were less effective in improving incidental symptoms. The cause is yet unknown. Kampo formulations have an advantage with respect to the overall condition of the patients. Kampo formulations have almost the same accelerated effects against rashes and incidental symptoms as antimicrobial agents. However, the same kinds of Kampo formulations should not be administered for prolonged periods, if incidental symptoms and/or rash have not improved.

In conclusion, we now have a greater understanding of Kampo medicines and bacteria, or utility of Kampo medicines, but the mechanisms of pharmacological activities of Kampo crude drugs are yet sometimes very complex.

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