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(*As of February 2019*)

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Original Article

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A survey on awareness of the "finger-tip unit" and medication guidance for the use of topical steroids among community pharmacists

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Summary **Atopic dermatitis (AD) is a common chronic, pruritic inflammatory skin condition. AD is most commonly treated with topical corticosteroids, and the finger-tip unit (FTU) should be used as a guideline for the amount to be used per application. In this study, we investigated the adequacy of pharmacists' instructions on the amount of topical steroids to be applied and the way in which they enhance the effect of pharmaceutical interventions. A self-administered anonymous questionnaire was distributed using QLifePro to 300 pharmacists working in insurance pharmacies that filled at least one dermatologist's prescription per month on average in Japan. Out of 300 pharmacists, 196 (65.3%) recognized the Japanese Dermatological Association's 2016 guidelines for the treatment AD, 107 (35.6%) gave instructions using the FTU as an index of external dose of topical steroids, 157 (52.3%) explained the amount of steroid application using an index other than FTU, and 61 (38.9% of 157) had inadequately instructed AD patients to apply steroids as a thin layer. Pharmacists who had read the guidelines for AD tended to give an appropriate instruction using FTU as an index of external dose of topical steroids ($p < 0.001$). We found that many pharmacists in pharmacies gave inadequate instructions on the amount of topical steroid application and deviated from the guidelines for AD, mainly because of inadequate knowledge of the guidelines.**

Keywords: Pharmacist, topical steroid, instruction, topical application, finger-tip unit

1. Introduction

Atopic dermatitis (AD), included in the eczema/dermatitis group, is a skin disorder characterized by chronic inflammation and pruritus (*1*). The number of AD patients is increasing, especially in developed countries. AD affects people of all ages, and its prevalence in children worldwide is reported to be as high as 20% (*2*).

Therapeutic options for chronic AD abound, with the mainstay of therapy being topical corticosteroids and topical calcineurin inhibitors. Topical steroids in Japan are classified into 5 ranks from "weak" to "strongest," and a steroid of an appropriate rank is used in accordance with the severity of cutaneous inflammation (*1*). In addition, a topical steroid should be administered at a necessary and sufficient dose; even when a higher rank steroid is prescribed because of exacerbation, if the patient spreads it too thinly, it will not produce the anticipated effect.

According to the 2016 guidelines for the treatment of AD issued by the Japanese Dermatological Association, the finger-tip unit (FTU) should be used as an index of the external dose of topical steroid. One FTU is the quantity of ointment that will be pushed out of a tube with a mouth 5 mm in diameter onto the region from the distal interphalangeal joint to the distal end on the pulp side of an adult's forefinger and is equivalent to approximately 0.5 g; it can cover 2 palms

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of an adult (approximately 2% of body surface area). One study found that low adherence was associated with prescriptions with unclear instructions about "how much product should be used" and "the area of spread" (*3*). Another study reported that dermatologic patients applied only 35% of the expected individualized dosages on average (*4*), so the dose of topical steroid is critical information for patients. In fact, provision of clear FTU-based instructions in the use of topical steroid optimized the topical dose and spreading area, and hydrocortisone uptake by the stratum corneum was significantly improved (*5*). Therefore, we believe that instructions based on the FTU concept are necessary for patients to get the full effect of steroids.

The Pharmacists Act in Japan states that pharmacists are responsible for not only the safety and efficacy after drug use, but also providing patients with necessary dosing information (*6*). However, it is still unclear how community pharmacists across Japan provide clear instructions to patients on the use of topical steroids. In this study, we investigated whether community pharmacists give patients adequate instructions on the amount of topical steroid to be applied.

2. Materials and Methods

2.1. *Questionnaire survey*

The survey was undertaken from July 8 to 12, 2016. The survey targeted 300 community pharmacists in Japan whose pharmacies had filled at least one dermatologist's prescription in a month. A web-based, self-administered, anonymous questionnaire was used *via* QLifePro, a web service for healthcare professionals. This study was approved by the research ethics committee of Keio University Faculty of Pharmacy (approval number: 160613-2). The questionnaire consisted of 17 questions in various formats: yes/no questions, multiple response questions, and open-ended questions. The questions covered the following: 1) screening questions and basic characteristics, 2) pharmacist's knowledge of topical steroid therapy and steroid phobia, 3) pharmacist's experience of providing medication instruction and information on topical steroid therapy to patients, and 4) pharmacist's experience of being provided limited information on topical steroid therapy by the doctor.

2.2. *Statistical analysisb*

All analyses were performed using IBM® SPSS® Statistics 23 software, and χ^2-tests were used to assess relationships between questions.

3. Results

3.1. *Basic characteristics*

The basic characteristics of the targeted community pharmacists are shown in Figure 1. Most pharmacists (86% of the total) were in their 30s, 40s, or 50s. In addition, the most common pharmacy scale (27.7%) was 2-5 chain stores.

3.2. *Level of understanding of the guidelines*

To clarify whether Japanese community pharmacists have enough knowledge of dermatological treatments, question 1 asked about their level of understanding of the guidelines for the treatment of AD issued by the Japanese Dermatological Association. We learned that 23.0% of pharmacists had read the guidelines completely, 42.0% had read them partially, and 35.0% had never read them (Figure 2).

3.3. *Medication instruction and information provision on topical steroid therapy*

Question 2 asked pharmacists about the importance they place on various aspects of the steroid administration guidance when counseling patients. Eighty-four percent of pharmacists said the amount of topical steroid was either "very important" or "somewhat important," and it was the fourth most emphasized item after rank

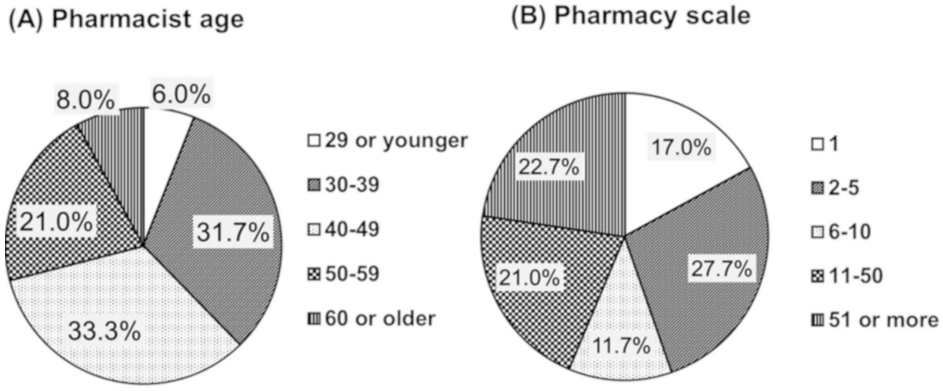

Figure 1. Basic characteristics of targeted community pharmacists (%, *n* = 300)**.**

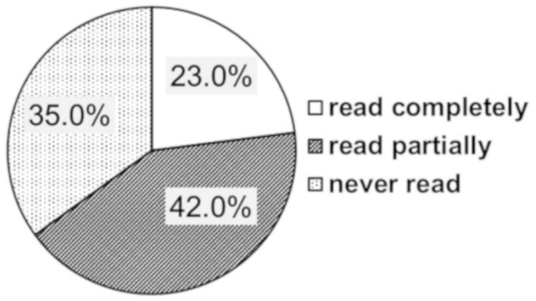

Figure 2. Degree of understanding of the guidelines. The answer for Q1 in the questionnaire is shown. [Q1: Have you ever read the Japanese guidelines for AD (16th edition or older)?] (%, *n* = 300)**.**

of steroid (93.0%), application sites (92.4%), and indication (89.7%) (Figure 3).

3.4. *Knowledge of FTU and the actual instruction in the use of topical steroid*

In response to question 3, regarding knowledge of the FTU, 196 (65.3%) pharmacists said they were very familiar with the FTU and were able to explain it to patients (Figure 4A). Of these 196 pharmacists, however, only 107 (54.6%) said that they always or usually explained dosage based on FTU to patients who were prescribed a steroid for the first time (question

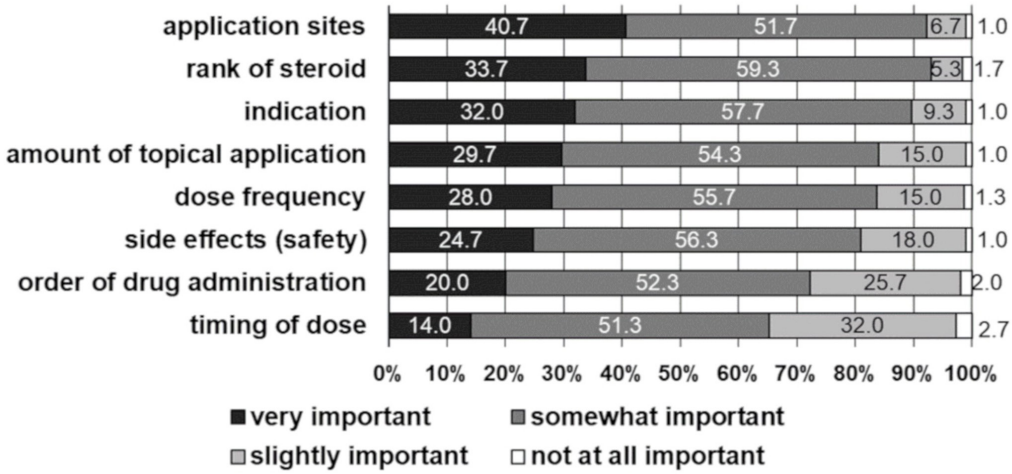

Figure 3. Medication instruction and information provision on topical steroid therapy. The answer for Q2 in the questionnaire is shown. [Q2. How much importance do you give to the following items in the drug administration guidance: rank of steroid, application sites, indication, amount of topical application, dose frequency, side effects (safety), order of drug administration, and timing of dose?] (%, *n* = 300)**.**

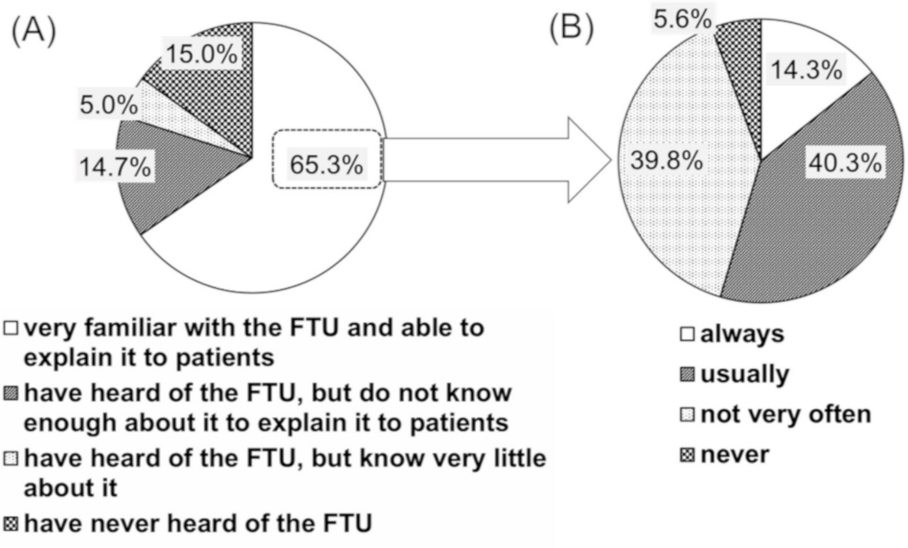

Figure 4. Knowledge of FTU. (A) The answer for Q3 in the questionnaire is shown. [Q3. Do you know the FTU (finger-tip unit)?] (%, *n* = 196). **(B)** The answer for Q4 in the questionnaire is shown. [Q4. (If you answered "Very familiar with the FTU and able to explain it to patients" in Q3) Do you explain the FTU to patients who have been prescribed a steroid for the first time when giving drug administration guidance?] (%, *n* = 196).

4, Figure 4B). When instructing the patient about the amount of topical steroid to apply, 157 (52.3%) pharmacists had explained the amount of topical steroid with a method other than the FTU (question 5, Figure 5A). Of the 143 pharmacists who responded that they had not used another method to explain the amount of topical steroid, only 45 (31.5%) pharmacists explained it based on the FTU (Figure 5B). Among pharmacists who had explained the amount of topical steroid with a method other than FTU, 61 (38.9%) pharmacists gave AD patients inadequate instructions to apply steroids thinly (Figure 5C).

3.5. *Relationship between comprehension of the guidelines and the appropriate instruction in the use of topical steroid*

Comprehension of the guidelines significantly increased with the rate of giving instruction based on the FTU in the cross-tabulation of these two variables ($p < 0.001$): 80.0% of pharmacists who had read the guidelines completely said they "always" or "usually" used the FTU in their instruction, compared with 51.1% of pharmacists who had read them partially (Figure 6). Comprehension of the guidelines also significantly correlated with the frequency of confirming the dose administered by the patient ($p < 0.001$): 69.5% of pharmacists who had read the guidelines completely said they "always" or "usually" checked the amount of topical steroids, compared with only 24.8% of pharmacists who had never read them (Figure 7).

4. Discussion

Currently, therapies for AD that follow the guidelines issued by the Japanese Dermatological Association enable the control of symptoms in most patients, to the extent that these symptoms do not interfere with daily

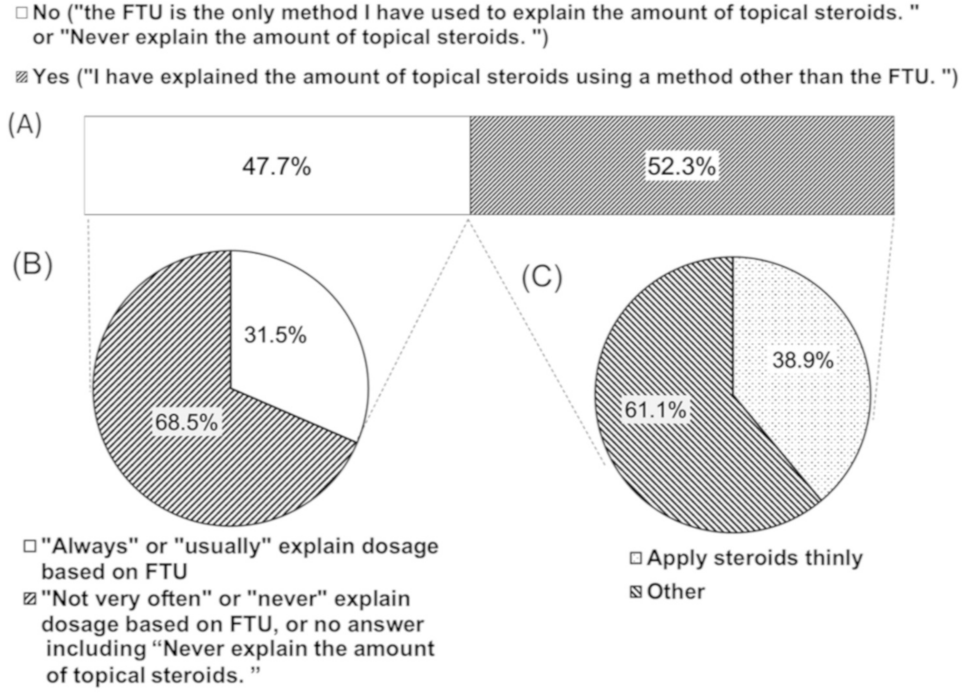

Figure 5. Explanation of the amount of topical steroid with a method other than FTU. (A) The answer for Q5 in the questionnaire is shown. [Q5. Have you ever explained the amount of topical steroid with a method other than the FTU?] (%, n = 300). **(B)** Among pharmacists who answered "Yes" to Q5 (A), the breakdown of answers to Q4 (%, n = 143). **(C)** Among pharmacists who answered "No" to Q5 (A), the detail drug administration guidance given (%, n = 157).

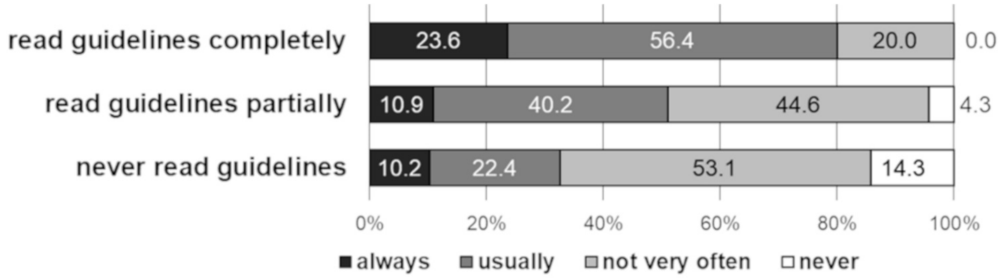

Figure 6. Relationship between the comprehension of the guidelines for the treatment of AD issued by the Japanese Dermatological Association and the rate of giving instruction based on the FTU (Q1 and Q4) (%, n = 300).

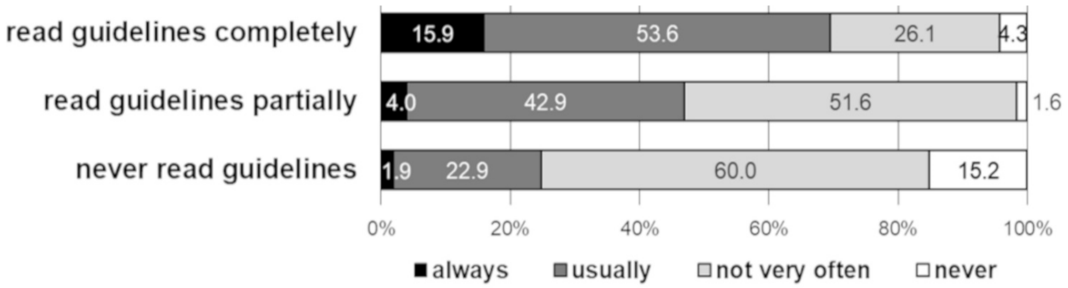

Figure 7. Relationship between the comprehension of the guidelines for the treatment of AD issued by the Japanese Dermatological Association and the frequency of confirming the dose of topical steroids administrated by the patient (Q1 and Q12) [Q12. Do you confirm whether the dose (not frequency) of topical steroids administrated by the patient was correct or not in a medication guidance for a patient who has used topical steroids continuously?] (%, *n* = 300).

activities. However, only some of the doctors who claim to be allergists have a professional qualification in this specialization. It is known that less than half of these 'allergists' have the latest edition of the clinical practice guidelines, and some doctors do not follow the treatment guidelines (*1*). Thus, the role of community pharmacists has become increasingly important, especially when providing instructions on topical steroid application.

In this study, we surveyed the pharmacists' awareness of providing instruction on the amount of topical steroid to be applied. We found that only about 15% of pharmacists explained the amount of topical steroid on the basis of FTU and some inadequate instructions would be provided, even though over 80% of pharmacists said that it was important to emphasize the amount of topical steroids (Figures 3-5). Though the detail of the dose of steroids was unclear, applying steroids too thinly seems to lead insufficient topical amount and insufficient effects.

We also found that pharmacists' level of understanding of the guidelines significantly correlated with their rate of giving instruction based on the FTU and their frequency of confirming the dose administered by the patient (Figures 6 and 7). This suggests that pharmacists' level of understanding of the guidelines should be well correlated with their ability to carry out some of the functions of a pharmacist.

In conclusion, we found that Japanese community pharmacists thought that counseling patients about the amount of topical steroids is important, but some provided inadequate instructions, failing to use the concept of the FTU. Also, the comprehension of the guidelines among Japanese community pharmacists was significantly correlated with their rate of giving instructions based on the FTU and their frequency of checking the amount of topical steroids. We suggest that more Japanese community pharmacists become familiar with the guidelines and use the knowledge when counseling patients about their medication for AD. In the next issue, we will reveal the factors related to inadequate medication guidance for the use of topical steroids.

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Original Article

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Additive effects of Kothala himbutu (*Salacia reticulata*) extract and a lactic acid bacterium (*Enterococcus faecalis* YM0831) for suppression of sucrose-induced hyperglycemia in an *in vivo* silkworm evaluation system

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Summary **Using a silkworm evaluation system, we previously evaluated various substances that suppress postprandial hyperglycemia. *Enterococcus faecalis* YM0831, a lactic acid bacterium that inhibits glucose uptake by the human intestinal Caco-2 cell line, exhibited hyperglycemia-suppressing effects in the silkworm system. In the present study, we found that Kothala himbutu (*Salacia reticulata*) extract, a traditional medicine containing α-glucosidase inhibitors, suppressed sucrose-induced hyperglycemia in the silkworm system. Moreover, combined oral administration of lactic acid bacteria YM0831 with Kothala himbutu extract had stronger suppressive effects on sucrose-induced hyperglycemia than single administration of either component. These findings suggest that the silkworm system provides a simple way to evaluate the effects of supplements on the suppression of blood glucose level induced by sucrose ingestion.**

Keywords: Kothala himbutu, lactic acid bacteria, *Salacia reticulata*, silkworm, hyperglycemia

1. Introduction

Medical treatments against lifestyle diseases are often problematic and must be continued for a long time. Therefore, establishing strategies to prevent the development of lifestyle-related diseases is important for human health care. Diabetes mellitus is a lifestyle disease characterized by a high blood glucose level. Long-term hyperglycemia leads to serious complications, including retinopathy, nephropathy, and neuropathy. Excess intake of sucrose is a proposed risk factor for the onset of diabetes (*1,2*). Sucrose, a common sweetener in various foods and beverages, is degraded to glucose and fructose by α-glucosidase in the intestine and absorbed into the blood. Therefore, suppression of the sucret alose degradation or absorption process is expected to prevent the onset of diabetes. Combinations of compounds that have different modes of action are expected to more effectively prevent diabetes.

Hypoglycemic agents are generally evaluated by *in vivo* experiments in animal models. Evaluation methods using mammals, however, are not only expensive, but are also associated with ethical issues with respect to animal welfare. We previously reported that the silkworm, an insect larva, can be used to evaluate the effects of different hypoglycemic agents (*3-9*). Ingestion of sucrose rapidly increases the blood glucose level in silkworms, and acarbose and voglibose, α-glucosidase inhibitors used clinically for human patients, also suppress the increase in silkworms (*3*). We demonstrated that a lactic acid bacterium, *Enterococcus faecalis* YM0831, suppresses sucrose-induced hyperglycemia in both silkworms and humans (*10*). Yogurt produced by the lactic acid bacterium YM0831 also has suppressive effects in humans (*10*). Therefore, the YM0831 strain is expected to be useful to suppress sucrose-induced hyperglycemia. This bacterium inhibits glucose-uptake

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into the human intestinal Caco-2 cell line, suggesting that one mechanism by which this lactic acid bacterium suppresses hyperglycemia is inhibition of glucose uptake by the intestine.

Kothala himbutu (*Salacia reticulate*, SR) extract is a traditional medicine used for diabetes patients in Asia. Hot water extract of SR is reported to inhibit α-glucosidase and to suppress sucrose-induced hyperglycemia in humans (*11*). α-Glucosidase inhibitors, such as salacinol and kothalanol, were isolated from SR extract (*12-14*). Therefore, SR extract acts to suppress sucrose-induced hyperglycemia through its effects to inhibit α-glucosidase.

Acarbose and voglibose are prescribed as anti-diabetic agents. Daily use of these medicines for preventing diabetes is not recommended for healthy humans. Therefore, it is highly desirable to screen for foods and supplements that could suppress sucrose-induced hyperglycemia. In the present study, we used the silkworm model to evaluate whether the lactic acid bacterium YM0831, a glucose uptake inhibitor, and SR extract, an α-glucosidase inhibitor, would have additive effects to suppress hyperglycemia.

2. Materials and Methods

2.1. *Reagents*

Freeze-dried powder of Kothala himbutu (*Salacia reticulata*) hot water extract was obtained from Snowden Co., Ltd. (Tokyo, Japan). The salacinol content in the freeze-dried powder was determined to be 0.36% using a liquid chromatography-mass spectrometry method at Japan Food Research Laboratories (Tokyo, Japan) (*15*).

2.2. *Culture of lactic acid bacteria*

Enterococcus faecalis YM0831 was anaerobically cultured in MRS medium at 30˚C for 2 days. Bacterial cells were harvested by centrifuge and the wet pellet was used as a sample.

2.3. *Silkworm rearing conditions and sucrose tolerance test*

Silkworms were reared according to a previously reported method (*4,16*). The silkworms were tested for sucrose tolerance as previously reported (*3*). Test samples were mixed in artificial diet containing 10% glucose or 10% sucrose. After feeding for 1 h, the glucose level in the silkworm hemolymph was measured using a glucometer (Accu-Chek, Roche).

2.4. *Statistical analysis*

All experiments were performed at least twice. The significance of differences was calculated using a two-tailed Student's t-test at the significance level of α = 0.05.

3. Results

3.1. *Suppression of sucrose-induced hyperglycemia by Kothala himbutu (Salacia reticulate, SR) extract*

We first tested whether SR extract suppresses sucrose-induced hyperglycemia in silkworms. The blood glucose levels were much lower in silkworms that ingested the SR extract compared with the control group, which did not ingest SR extract (Figure 1A). The SR extract, however, did not affect glucose-induced hyperglycemia in silkworms (Figure 1B).

3.2. *Additive effect of E. faecalis YM0831 and SR extract on sucrose-induced hyperglycemia in silkworms*

We previously reported that a sucrose-induced increase in the blood glucose level was suppressed by the ingestion of *E. faecalis* YM0831 in both silkworms and humans (*10*). The suppressive effect of this lactic acid bacterium is due to its activity to inhibit glucose transport in the intestine (*10*). Therefore, the mechanisms by which SR extract and *E. faecalis* YM0831 inhibit sucrose-induced hyperglycemia are different. We used the silkworm evaluation system to test our hypothesis that these substances would have an additive effect. Compared with SR extract (0.31% in diet) alone, administration of both lactic acid bacteria YM0831 and SR extract induced a greater decrease in the blood glucose level in silkworms that ingested sucrose (Figure 2A). Further, the blood glucose level was much lower in silkworms

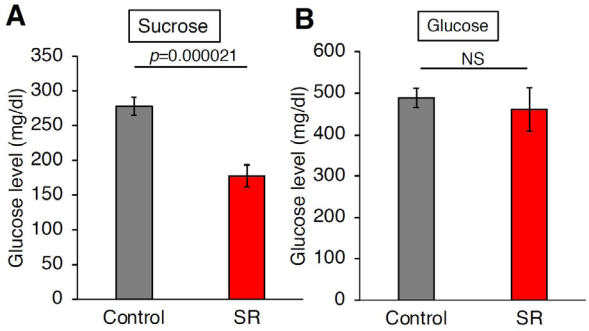

Figure 1. Effects of SR extract on dietary sucrose-induced increases in glucose levels in silkworm hemolymph. A. Silkworms were fed a diet containing 10% (w/w) sucrose with 1% (w/w) SR extract (SR, $n = 20$) or without SR extract (Control, $n = 28$) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean ± SEM. Statistically significant differences between the control and testing groups were evaluated using Student's *t*-test. **B.** Silkworms were fed a diet containing 10% (w/w) glucose with 1% (w/w) SR extract (SR, $n = 12$) or without SR extract (Control, $n = 14$) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean ± SEM. Statistically significant differences between control and testing groups were evaluated using Student's *t*-test. NS: not significant.

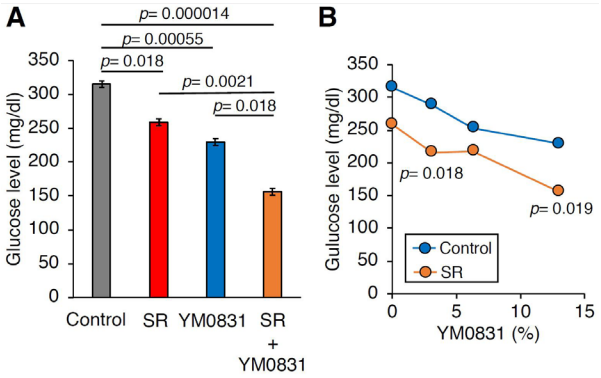

Figure 2. Combined effects of SR extract and Enterococcus faecalis YM0831 on dietary sucrose-induced increases in glucose levels in silkworm hemolymph. A. Silkworms were fed a diet containing 10% (w/w) sucrose (Control, n = 14), the 10% sucrose diet with 13% (w/w) *E. faecalis* YM0831 (*E. faecalis*, n = 14), the 10 % sucrose diet with 0.3% (w/w) SR extract (SR, n = 14), or the 10% sucrose diet with 13% (w/w) *E. faecalis* YM0831 and 0.3% (w/w) SR extract (*E. faecalis* + SR, n = 10) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean ± SEM. Statistically significant differences between control and testing groups were evaluated using Student's t-test. **B.** Silkworms were fed a diet containing 10% (w/w) sucrose and 0-13% (w/w) *E. faecalis* YM0831 with 0.3% (w/w) SR extract (SR; orange line) or without SR extract (Control; blue line) for 1 h. Glucose levels in the silkworm hemolymph were measured. Data represent mean ± SEM. Statistically significant differences between control and testing groups were evaluated using Student's t-test. n = 10-14.

that ingested viable lactic acid bacteria YM0831 with SR extract (0.31% in diet) than in silkworms ingesting the bacteria without SR extract (Figure 2B).

4. Discussion

In the present study, using silkworms as a model animal, we showed that combined administration of lactic acid bacteria *E. faecalis* YM0831 and SR extract had additive effects to suppress sucrose-induced hyperglycemia. To our knowledge, this is the first report to show additive effects of anti-hyperglycemia substances that act by different mechanisms in an invertebrate evaluation system.

SR is a plant used as a traditional medicine in the Indian subcontinent. Hot water extract of SR has α-glucosidase inhibitory activity (*13*) and suppresses postprandial hyperglycemia in mammalian animals, including humans (*11*). SR extract suppresses sucrose-induced hyperglycemia, but not glucose-induced hyperglycemia, in rats (*13*). These findings suggest that the suppression of hyperglycemia by SR extract is due to its effects to inhibit the degradation of sucrose in the intestine. Our findings indicated that SR extract also suppressed sucrose-induced hyperglycemia, but not glucose-induced hyperglycemia, in silkworms.

E. faecalis YM0831 was screened using the silkworm evaluation system as a functional lactic acid bacterium that suppresses sucrose-induced hyperglycemia (*10*). This lactic acid bacterium also suppressed sucrose-induced hyperglycemia in humans (*10*). Moreover, it inhibited glucose-uptake by the human intestinal Caco-2 cell line (*10*). Therefore, we consider that the inhibition of sugar absorption from the intestine is the likely mechanism for this bacterium to suppress a sucrose-induced increase in the blood glucose level. In this paper, we demonstrated that combined administration of this lactic acid bacteria and the SR extract had additive effects to suppress sucrose-induced hyperglycemia. On the basis of these results, we propose that combinations of foods or supplements with α-glucosidase inhibitory activity and glucose transport inhibition activity may efficiently suppress the postprandial increase in blood glucose and may contribute to prevent the lifestyle diseases such as diabetes and obesity.

In conclusion, the findings of the present study suggest that SR extract and *Enterococcus faecalis* YM0831 have additive effects to inhibit postprandial sucrose-induced increases in blood glucose and that silkworms are a useful animal model for testing the additive effects of anti-hyperglycemia substances.

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Original Article

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Status of use of protease inhibitors for the prevention and treatment of pancreatitis after endoscopic retrograde cholangiopancreatography: An epidemiologic analysis of the evidence-practice gap using a health insurance claims database

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Summary **Existing evidence suggests that protease inhibitors (PIs) used to prevent or treat pancreatitis after endoscopic retrograde cholangiopancreatography (ERCP) are ineffective, and their use is not recommended by clinical practice guidelines. However, in Japan, PIs are administered with the aim to prevent or treat post-ERCP pancreatitis. This study aimed to clarify the gap between guideline recommendations and contents of practice. We used the health insurance claims database of Japan Medical Data Center. Among patients who had undergone ERCP, those with acute pancreatitis or post-ERCP pancreatitis recorded in claims as disease names were defined as post-ERCP pancreatitis patients. The study period was divided into three terms according to the date of publication of clinical practice guidelines for acute pancreatitis. Among 2,945 patients who had undergone ERCP, 2,847 were eligible for analysis. Of these, 1,375 (48.3%) patients had claims with pancreatitis recorded as the disease name; PIs were prescribed to 1,238 (90.0%). Rates of prescription of PIs were 72.3% in 2005-07, 70.9% in 2008-09, and 83.6% in 2010-15, showing a significant increase ($p < 0.001$). In conclusion, PIs are administered in clinical practice in Japan for the purpose of preventing or treating pancreatitis, with an increasing trend in prescription in recent years.**

Keywords: Protease inhibitor, pancreatitis, endoscopic retrograde cholangiopancreatography (ERCP), evidence-practice gap, health insurance claims database

1. Introduction

Since 1991, evidence-based medicine (EBM) has been adopted in various clinical fields along with an increase in social awareness that seeks high-quality medical care (*1*). However, it is not always the case that evidence-based medical care is practiced in clinical settings (*2-7*). One study reported that guideline-recommended medical care is provided to roughly 50% of adult patients (*8*). In Japan, rates of implementation of some guideline-recommended treatments are reported to be roughly 25% (*3,7,9*). Even results from multiple randomized controlled trials (RCTs) have gained little attention among general clinicians; in fact, those results are not necessarily reflected in actual clinical activities (*10*). In other words, there is an "evidence-practice gap." In order to fill this gap, active communication between clinicians and clinical researchers, *i.e.*, those who create evidence, is necessary (*11*).

At present, several RCTs (*12-14*) and systematic reviews (*15-17*) have concluded that the administration of protease inhibitors (PIs) prior to endoscopic retrograde cholangiopancreatography (ERCP) has no preventive effect against post-ERCP pancreatitis. The first edition of the Japanese (JPN) Guidelines for the Management of Acute Pancreatitis (hereafter, "JPN guidelines"), issued in 2003 by the Japanese Society for Abdominal Emergency Medicine and Japan Pancreas Society (*18*), states that clinical usefulness of PIs in mild or moderate acute pancreatitis is unclear.

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Nonetheless, PIs are widely used in Japan (*19*). The third edition of the JPN guidelines, which was developed by the Japanese Society for Abdominal Emergency Medicine, Research Committee on Intractable Diseases of Pancreas supported by Health and Labour Sciences Research Grants of the Ministry of Health, Labour and Welfare of Japan, Japanese Society of Hepato-Biliary-Pancreatic Surgery, Japan Pancreas Society, and Japan Radiological Society, states that administration of PIs for acute pancreatitis could be considered an option, albeit on insufficient grounds (*20*). Moreover, the fourth edition states that the effectiveness of PIs for "improving the prognosis and rate of complications associated with acute pancreatitis has not been clearly proven" by clinical studies and avoiding routine use is suggested (*21*). Furthermore, according to this edition, active evidence suggests a lack of efficacy of PIs administered prior to ERCP for the purpose of preventing the onset of post-ERCP pancreatitis (*21*). However, updated data from observational studies of actual clinical settings suggest the limited use of evidence (*19,22*). In 2015, the Japan Pancreas Society published clinical practice guidelines for post-ERCP pancreatitis (*23*), which did not recommend the use of PI because it does not prevent post-ERCP pancreatitis (*5*) based on several RCTs (*13,14*) and meta-analyses (*15,16*). However, it also states, without citation, that PIs – which are covered by insurance – are generally administered as a matter of principle if patients are diagnosed with acute pancreatitis.

Based on the evidence currently available, the value of PIs for treating acute pancreatitis or preventing the development of post-ERCP pancreatitis is low. From the viewpoint of a clinician, however, it is difficult to judge whether or not to prescribe PIs, since descriptions regarding their use in clinical practice guidelines are inconsistent. This study aimed to investigate the status of PI use for preventing or treating post-ERCP pancreatitis in Japan and clarify factors associated with the gap between guideline recommendations and actual clinical practice.

2. Materials and Methods

2.1. *Study design*

Japan has a universal health insurance system, and healthcare services are provided through public insurers largely divided into the following three categories: Employee Health Insurance, National Health Insurance administered by local governments, and the Medical Care System for the Advanced Elderly (aged ≥ 75 years). Japanese citizens (*i.e.*, the insured) pay insurance premiums to their medical insurers, and when they receive medical services from authorized insurance medical institutions such as hospitals, clinics, and pharmacies, they accept responsibility for some costs, while the remaining expenses are covered by payments of medical service fees made by the medical insurer through the examination and payment agency. All residents of Japan are required to subscribe to either one of the public insurances, and their activities (*i.e.*, medical services they receive) can be traced, unless they cancel their insurance, no matter where or how many medical institutions/pharmacies they visit.

The present study used data registered from February 2005 through January 2015 (extracted in August 2015) in the Japan Medical Data Center (JMDC) Claims Database (developed by the Japan Medical Data Center Co., Ltd.). While the JMDC Claims Database only contains data of company employees (aged ≤ 74 years) and their families, they can be used to examine disease prevalence and incidence in this population by matching claims data with health insurance enrollment data. Moreover, health insurance claims of all enrolled individuals are aggregated by name in an anonymized state and are thus traceable, even if they transfer to another hospital or visit multiple facilities. Thus, the actual state of medical services provided, not only by large-scale medical facilities but also medium-sized to small hospitals, as well as medical clinics with beds, can be assessed (*23*). This allows us to understand better the true state of real-world medical care in a continuous fashion, as the database provides complete data (*i.e.*, administrative data) of all individuals enrolled in health insurance unions (*24*).

2.2. *Eligibility criteria*

Patients who underwent ERCP were defined as those for whom ERCP was performed with or without subsequent biliary or pancreatic procedures (procedures 1 and 2; Table 1). Among these, those with "acute pancreatitis" (disease code: K85) or "post-ERCP pancreatitis" (disease code: K918 or K85) as recorded in claims were defined as patients with post-ERCP pancreatitis and were included in this study.

2.3.*Data items and extraction methods*

Sex, age, number of beds, date of claims, type of PI used (by generic name), and the method of PI administration (intravenous drip infusion) were used as patient factors. Among the names of diseases recorded in claims, we could not distinguish between severe acute pancreatitis and acute pancreatitis, as the same disease code (K85) was used for both. Therefore, when claims containing the disease name "acute pancreatitis" or "post-ERCP pancreatitis" also contained at least one of the severe disorders listed in Table 2, these patients were assumed to be severe acute pancreatitis patients. Given the possibility that the publication of clinical practice guidelines might have altered treatment practice, analyses were performed by dividing the study period

Table 1. Specific codes for ERCP examination and procedures

Procedure	Name	Specific code
1	Endoscopic retrograde cholangiopancreatography	D308 + (cholangiography or pancreatography or cholangiopancreatography)
2	Endoscopic nasobiliary drainage	K682-3
	Endoscopic cholangiography and stone removal	K685 or K6851 or K6852
	Endoscopic biliary balloon dilation	K686
	Endoscopic papillotomy	K687 or K6871 or K6872
	Endoscopic biliary stenting	K688
	Endoscopic pancreatic stenting	K708-3
	Endoscopic pancreatic pseudocyst drainage	K7071
	Endoscopic pancreatic stone removal (transduodenal sphincterotomy)	K6992

ERCP, endoscopic retrograde cholangiopancreatography.

Table 2. Indicators for suspected (presumed) severe acute pancreatitis patients

Name	Specific code
Maintenance or sustained dialysis	J038 or J038-2
Artificial respiration	J045
Angiography	E002
Abdominal paracentesis	J010 or J021
Disseminated intravascular coagulation	D65
Nasoenteric feeding	J120
Use of blood products	K920
Organ failure	R688
Shock	A419 or R571 or R579 or R570
Respiratory failure	J960 or J969 or R090
Renal failure	N170 or N178 or N179 or N19
Gastrointestinal hemorrhage	K921 or K922

into the first (2003-2007), second (2008-2009), and third (2010-2015) terms, according to the year of publication of the first (2003), second (2007), third (2009), and fourth (2015) editions. The identification code (ID) of each medical institution and hospital size (number of beds) were extracted as medical institution factors. Specifically, usage status was assessed according to the number of beds of < 200, ≥ 200 and < 500, and ≥ 500.

2.4. *Statistical analysis*

Proportions of patients for whom PIs were used were determined for those who developed post-ERCP pancreatitis and those who did not. Univariate analyses (Chi-square test, *etc*.) were performed by patient factors and severity. Subgroup analyses were performed to examine whether the trend for PI use differs by time of practice (*i.e*., first, second, and third terms) and number of beds, and trends were examined by the Cochrane-Armitage test. To examine factors associated with PI use, multiple logistic regression analysis was performed with severe patients (Model 1), post-ERCP pancreatitis (Model 2), and ERCP procedure (Model 3) as explanatory variables, in addition to sex, age, and number of beds. Moreover, the variance inflation factor (VIF) was calculated to examine multicollinearity of variables, and goodness-of-fit of each model was evaluated with the Hosmer-Lemeshow test. All tests were two-tailed, and $p < 0.05$ was considered statistically significant. All analyses were performed using Stata/SE 14 (StataCorp LLC, TX, USA) (*25*).

2.5. *Ethical considerations*

This study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (Approval No. R 0838) and the Ethics Committee of Japanese Red Cross Wakayama Medical Center (No. 447).

3. Results

3.1. *Patient characteristics*

Among 3,204,575 insured individuals who comprised the observed population, 2,945 had undergone ERCP (1,897 men and 1,048 women; median age, 55 years) (Figure 1). Of these, 2,847 patients were subjected to analysis, excluding 98 who were considered ineligible due to missing data.

Among the 2,847 analyzed patients, 1,375 (48.3%) developed post-ERCP pancreatitis, while the remaining 1,472 (51.7%) did not. PIs were used in 1,238 (90.0%) of the 1,375 patients with post-ERCP pancreatitis, and 1,083 (73.6%) of the 1,472 patients without post-ERCP pancreatitis. Among those for whom PIs were used,

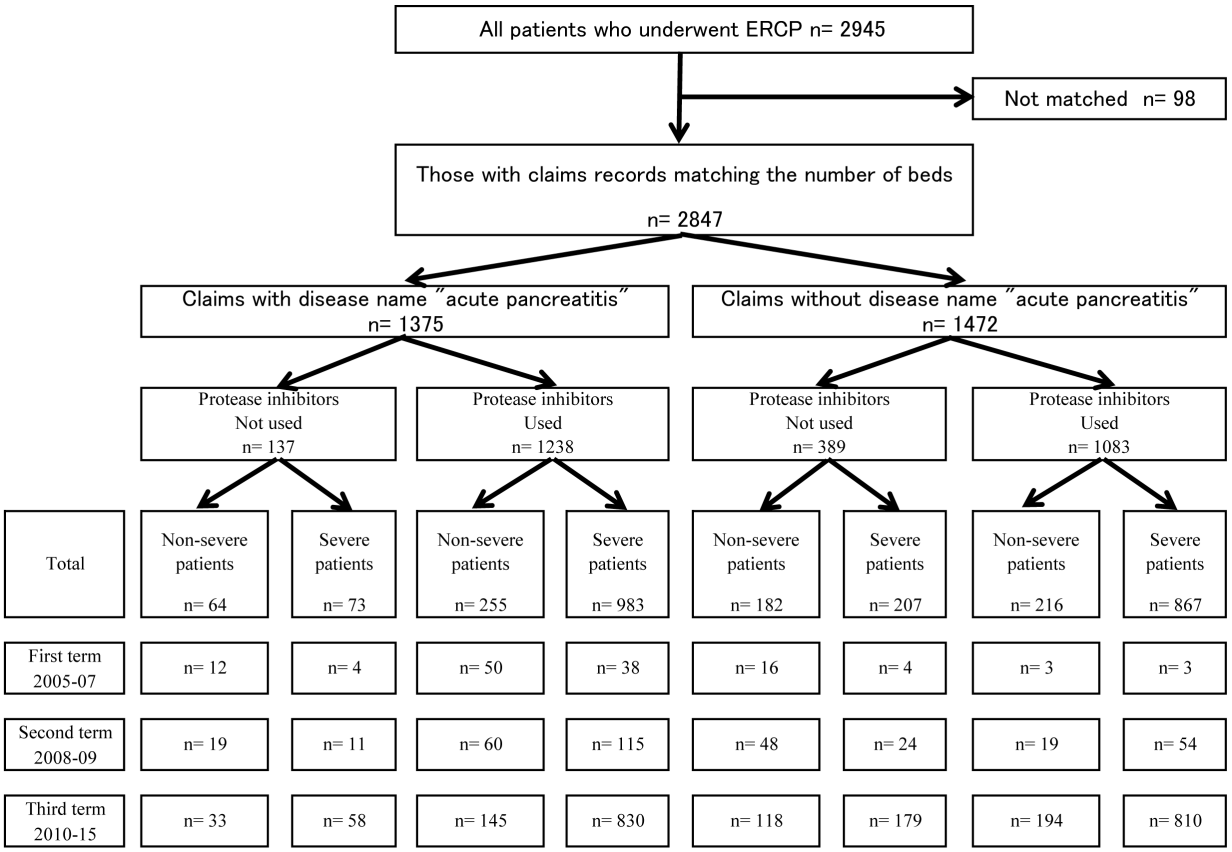

Figure 1. Relationships between patients who underwent ERCP, status of protease inhibitor use, and severity.

225 (20.6%) of the 1,238 patients with post-ERCP pancreatitis were non-severe patients, and 216 (19.9%) of the 1,083 patients without post-ERCP pancreatitis were non-severe patients.

3.2. *Analysis results of the frequencies of protease inhibitors using to prevent and treat pancreatitis after ERCP and multiple logistic regression analysis*

Table 3 shows the number of patients who underwent ERCP and changes in the number of PI prescriptions over time. The proportions of PI users for the first, second, and third terms according to the year of publication of the JPN guidelines were 72.3%, 70.9%, and 83.6%, respectively, showing a significant increase over time ($p < 0.001$), with a particularly marked increase from the second to the third term. Among severe patients, the proportions of PI users were 83.7%, 82.8%, and 87.4%, respectively, and although no significant increasing trend was observed ($p = 0.15$), the proportions of PI users remained high throughout the entire period. When the analysis was limited to non-severe patients, the proportions of PI users for each term were 65.4%, 54.1%, and 69.2%, respectively, with a significant increasing trend over time ($p = 0.003$), particularly from the second to the third term.

Table 4 shows the results of subgroup analysis of patients with post-ERCP pancreatitis. The rate of PI use significantly increased over time ($p = 0.004$). When the analysis was limited to severe or non-severe patients, an increase in the rate of PI use was observed, but changes were not significant ($p = 0.52$ and $p = 0.59$, respectively).

Table 5 shows the results of the subgroup analysis of patients without post-ERCP pancreatitis. The rate of PI use significantly increased over time ($p < 0.001$). When limited to severe or non-severe patients, the analysis revealed a significant increase in the rate of PI use in both severe and non-severe patients ($p = 0.001$ and $p < 0.001$, respectively).

Table 6 shows the proportion of PI users by hospital/clinic size. The proportion of PI users and severity showed a significant decreasing trend with an increasing number of beds.

Table 7 shows the results of multiple logistic regression analysis. In Model 1, the rate of PIs use was significantly more frequent among severe patients (odds ratio: 3.48 [95% confidence interval: 2.85-4.25]). In addition, the rate of PIs use was significantly less frequent at facilities with a higher number of beds. While the presence of post-ERCP pancreatitis was significantly associated with PI use (Model 2), no association was observed with ERCP procedure (Model 3). The results of the Hosmer-Lemeshow test were $p = 0.07$, $p = 0.21$, and $p = 0.01$ for Models 1, 2, and 3, respectively, with the highest VIF of 2.09.

Table 3. Number of patients who underwent ERCP and changes in the number of protease inhibitor prescriptions over time (overall)

Items	Overall	Severe patients	Non-severe patients
First term (2005-2007)			
No. patients undergoing ERCP	130	49	81
No. PIs prescribed	94	41	53
Proportion (%)	72.3	83.7	65.4
Second term (2008-2009)			
No. patients undergoing ERCP	350	204	146
No. PIs prescribed	248	169	79
Proportion (%)	70.9	82.8	54.1
Third term (2010-2011)			
No. patients undergoing ERCP	2,367	1,877	490
No. PIs prescribed	1,979	1,640	339
Proportion (%)	83.6	87.4	69.2
Total			
No. patients undergoing ERCP	2,847	2,130	717
No. PIs prescribed	2,321	1,850	471
Proportion (%)	81.5	86.9	65.7
P value for trend	< 0.001	0.15	0.003

ERCP, Endoscopic retrograde cholangiopancreatography; PI, Protease inhibitors.

Table 4. Number of patients who underwent ERCP and changes in the number of protease inhibitor prescriptions over time (with post-ERCP pancreatitis)

Items	Overall	Severe patients	Non-severe patients
First term (2005-2007)			
No. patients undergoing ERCP	104	42	62
No. PIs prescribed	88	38	50
Proportion (%)	84.6	90.5	80.6
Second term (2008-2009)			
No. patients undergoing ERCP	205	126	79
No. PIs prescribed	175	115	60
Proportion (%)	85.4	91.3	75.9
Third term (2010-2011)			
No. patients undergoing ERCP	1,066	888	178
No. PIs prescribed	975	830	145
Proportion (%)	91.5	93.5	81.5
Total			
No. patients undergoing ERCP	1,375	1,056	319
No. PIs prescribed	1,238	983	255
Proportion (%)	90.0	93.1	79.9
P value for trend	0.004	0.52	0.59

ERCP, Endoscopic retrograde cholangiopancreatography; PI, Protease inhibitors.

Table 5. Number of patients who underwent ERCP and changes in the number of protease inhibitor prescriptions over time (without post-ERCP pancreatitis)

Items	Overall	Severe patients	Non-severe patients
First term (2005-2007)			
No. patients undergoing ERCP	26	7	19
No. PIs prescribed	6	3	3
Proportion (%)	23.1	42.9	15.8
Second term (2008-2009)			
No. patients undergoing ERCP	145	78	67
No. PIs prescribed	73	54	19
Proportion (%)	50.3	69.2	28.4
Third term (2010-2011)			
No. patients undergoing ERCP	1,301	989	312
No. PIs prescribed	1,004	810	194
Proportion (%)	77.2	81.9	62.2
Total			
No. patients undergoing ERCP	1,472	1,074	398
No. PIs prescribed	1,083	867	216
Proportion (%)	73.6	80.7	54.3
P value for trend	< 0.001	0.001	< 0.001

ERCP, Endoscopic retrograde cholangiopancreatography; PI, Protease inhibitors.

Table 6. Relationship between hospital/clinic size and the number of protease inhibitor users

Hospital/ clinic size	Total number of patients	No. patients who used protease inhibitors (%)	No. of patients with severe pancreatitis	No. of patients who used protease inhibitors with severe pancreatitis (%)	Percentage of patients with severe pancreatitis to total number of patients
≤ 199	209	186 (89.0)	163	145 (89.0)	78.0
200-499	873	696 (79.7)	658	558 (84.8)	75.4
≥ 500	1,765	1,439 (81.5)	1,309	1,147 (87.6)	74.2
Total	2,847	2,321 (81.5)	2,130	1,850 (86.9)	74.8

Table 7. Results of multiple logistic regression analysis on protease inhibitor use

Explanatory variables	Model 1			Model 2			Model 3		
	Odds Ratio	95% Confidence Interval		Odds Ratio	95% Confidence Interval		Odds Ratio	95% Confidence Interval	
		Lower limit	Upper limit		Lower limit	Upper limit		Lower limit	Upper limit
Severe patients	3.48	2.85	4.25		N/A			N/A	
Post-ERCP pancreatitis		N/A		3.26	2.62	4.04		N/A	
ERCP procedure		N/A			N/A		1.22	0.99	1.50
Sex	1.10	0.89	1.35	1.11	0.90	1.36	1.09	0.89	1.34
Age	1.00	0.99	1.00	1.00	0.99	1.00	0.99	0.99	1.00
No. beds									
≤ 199		1.00 (Reference)			1.00 (Reference)			1.00 (Reference)	
200-499	0.50	0.31	0.80	0.67	0.42	1.08	0.48	0.30	0.76
≥ 500	0.57	0.36	0.90	0.86	0.54	1.37	0.54	0.34	0.84
Hosmer-Lemeshow test		$P = 0.07$			$P = 0.21$			$P = 0.01$	

N/A, Not applicable; ERCP, Endoscopic retrograde cholangiopancreatography.

4. Discussion

Existing evidence suggests that PIs used to treat patients with acute pancreatitis, or to treat or prevent the onset of post-ERCP pancreatitis, is ineffective. While such use is also not recommended by clinical practice guidelines, we found that 80% of patients who had undergone ERCP, and 65% of non-severe acute pancreatitis patients, were administered PIs in Japan. This is the first report to show the actual state of PI prescription related to ERCP using large-scale real-world data.

Sekimoto *et al*. conducted a questionnaire survey on clinical practice for acute pancreatitis among clinicians before and after publication of the JPN guidelines, and reported that the use of PIs for patients with mild pancreatitis significantly decreased after publication of the guidelines at the time; no such change was observed in the use of PIs for moderate to severe pancreatitis (*19*). Murata *et al*. showed that intravenous PI infusion was administered in 86% and 80% of patients with mild acute pancreatitis before and after publication of the first (2003) edition of the JPN guidelines, respectively, using the Diagnosis Procedure Combination (DPC) system (*22*). Moreover, around the time when the third (2009) edition was published, PIs were used in 60.8% of patients with mild pancreatitis, and while the frequency of use had decreased since the publication of the first edition, 82.9% of patients – when considering those with severe pancreatitis only – were using PIs (*22*). Based on these results, Murata *et al*. concluded that there has been no change in clinical practice policy among clinicians in the past few years, and PIs are still used according to the traditional philosophy. The results of these previous studies, which used different methods to evaluate the degree of penetration of clinical practice guidelines, did not differ greatly from the results of the present study, which targeted a wider range of hospitals/clinics without limiting the analysis to DPC hospitals.

In the present study, we initially expected that the use of PIs for the prevention or treatment of pancreatitis occurring due to ERCP examination might have decreased with revisions of the JPN guidelines. However, in reality, PIs were used at a higher rate than expected even in the past few years. A possible reason for the high number of PI prescriptions is the existence of "clinical inertia" for the evidence-practice gap. It is said that physicians cannot easily change a traditional medical practice because they have been a human behavior, clinical inertia. Barth JH *et al*. showed "The main barriers include awareness, familiarity and agreement with the contents" as reasons why clinical practice guidelines cannot be adhered, and it is difficult to correct in the correct direction because familiar act exists (*26*).

The present study examined how the date of publication of clinical practice guidelines and the number of beds were related to the rate of PI use. With respect to the date of publication of clinical practice guidelines, the use of PIs did not decrease with guideline updates or dissemination, but rather showed an increasing trend. With respect to the number of beds, we hypothesized that the use of PIs might show

a decreasing trend with an increasing number of beds, *i.e*., big hospitals with many acute patients, since there are not only many patients undergoing ERCP but also specialists who are highly interested in clinical practice guidelines. The results of the analysis revealed that, while the rate of PI use significantly decreased with an increasing number of beds, more than 80% of patients who had undergone ERCP were administered PIs. These findings suggest that the understanding of the JPN guidelines and implementation of the recommendations have not improved over time more than we had anticipated, irrespective of the hospital or clinic size.

While sex and age of patients did not contribute to PI use, a significantly higher use was observed among severe patients. Progression to a severe state is known to be triggered by the onset of post-ERCP pancreatitis following ERCP procedures (endoscopic papillotomy, endoscopic biliary stenting, or endoscopic pancreatic stenting), which by itself is associated with severe disease. Moreover, ERCP examination itself could cause post-ERCP pancreatitis, thereby advancing in severity (*21*). ERCP procedures and post-ERCP pancreatitis are thus likely to act as intermediate (or mediating) variables. In clinical studies, variables for regression analysis should be selected based on clinical judgments, since a misplaced sense of confidence in statistical methods could lead to the selection of clinically meaningless variables or overfitting of models (*27*). For these reasons, we considered Model 1 (severe patients) to be the most clinically meaningful. The results of the multiple logistic regression analysis revealed a trend of increased PI use in severe patients. The most recent version of the JPN guidelines recommends against the routine use of PIs, based on the evidence suggesting a lack of efficacy of PIs on acute pancreatitis (*21*). Moreover, the effect of PIs in patients with severe acute pancreatitis has been demonstrated in clinical studies with high bias risk (*17*), so the true effect of PIs remains unclear. The present study revealed an increasing trend in PI use among patients with post-ERCP pancreatitis and those with severe conditions, suggesting the possibility that JPN guideline recommendations relating to treatment have not fully permeated into the practice of clinicians.

There are some limitations to this study. First, the JMDC medical data bank targets individuals enrolled in health insurance unions for employees of large companies, *i.e*., a population comprising individuals of relatively high socioeconomic status. As the enrollees are mainly in their 30s to 50s, the number of cases in which ERCP examination was performed was low. Second, disease names recorded in claims data do not necessarily reflect the accurate names of diseases diagnosed. The method used in this study does not allow for distinction between patients with 'true' post-ERCP pancreatitis and those with acute pancreatitis 'recorded in claims as the disease name,' since this was assigned in order to prescribe PIs for the purpose of preventing the onset of post-ERCP pancreatitis. Third, as health insurance claims do not contain clinical information and examination data, we could not assess the severity of pancreatitis using these data (*28*). While the rate of post-ERCP pancreatitis advancing in severity is reportedly low at around 2% (*28*), two-thirds of patients in the present study were in severe condition, which was clearly high. However, regardless of the severity of pancreatitis, there is no evidence that supports the effectiveness of PIs, and the conclusion that these drugs are over-prescribed for pancreatitis in Japan stands firm.

In conclusion, the present study found that, despite that no evidence support effectiveness of PIs on post-ERCP pancreatitis prevention and treatment, and that clinical practice guidelines don't recommend the use, PIs are frequently prescribed to prevent or treat post-ERCP pancreatitis in Japan, with an increasing trend even in recent years. But the proportion of PI users and severity showed a significant decreasing trend with an increasing number of beds.

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Molecular characterization of multi-drug resistant coagulase negative cocci in non-hospital environment

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Summary **Antibiotic resistance crisis occasioned by sporadic appearance of multi-drug resistance (MDR) in human pathogens to clinically applied antimicrobials is a serious threat to global health. In this study, we investigated the drug resistant phenotype of Gram-positive cocci isolates from environment. *Staphylococcus capitis* and *Staphylococcus haemolyticus* colonies were isolated on mannitol-salt agar plates supplemented with tetracycline. Antibiotic susceptibility profile of the isolates *via* minimum inhibitory concentration (MIC) determination was examined. Isolates showed decreased sensitivity to clinically applied antimicrobial agents: tetracycline, kanamycin, erythromycin, norfloxacin, teicoplanin, and ampicillin. Genomic analysis demonstrated the presence of multiple antibiotic resistant genes in these bacteria, suggesting the origin of the multiple antimicrobials resistant phenotype. Tetracycline resistance of these isolates was transduced to *Staphylococcus aureus*-RN4220 strain. These findings indicate the presence of multiple antimicrobials resistant *S. capitis* and *S. haemolyticus* strain in a non-hospital setting. Moreover, the presence of plethora of genes responsible for MDR suggest that these strains could present potential threat to human health by serving as reservoir for lateral transference of antimicrobial resistance conferring foreign genetic elements to other clinically relevant pathogens.**

Keywords: MDR, foreign genetic elements, micro-broth dilution, infectious diseases, antimicrobial resistance determinants, antibiotic resistance crisis

1. Introduction

Rampant increase in drug resistance among human pathogens has hampered antimicrobial effectiveness and limited available therapeutic options. Estimated not less than 700,000 people die annually because of antimicrobial resistant infections and is expected to rise to 10 million annual deaths by 2050 costing global economy USD100 trillion if the current trajectory is not halted (*1*). Understanding the various processes associated with drug resistance may provide important insights into new preventative and therapeutic strategies against resistant infections. Antimicrobial resistance (AMR) determinants are present as transposons, integrons or plasmids; which are utility vehicles for genetic transference to other clinically relevant microbes. AMR is complicated by the horizontal gene transfer mechanisms vis-a-vis conjugation, transduction and transformation into clinically relevant pathogens (*2-4*). Considering the foregoing, accurate identification and proper antimicrobial profiling of pathogenic species are necessary for tracking the epidemiology and treatment of infected patients in health-care settings. The environment is a reservoir of AMR genes and mobile genetic elements that are actively involved in resistant gene mobilization and transfer (*5*). Environmentally-borne dissidents are key threats to human health because of the increasing relevance of zoonotic diseases and their importance in predicting outbreak of infectious diseases (*6-9*). Despite all efforts to understand and abrogate AMR, it is evident that all is not well and further

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efforts including scientific investigations are therefore warranted. Coagulase-negative *staphylococci* (CoNS) are gradually becoming important pathogens associated with nosocomial infections. *Staphylococcus capitis*, a sub-group of CoNS and a colonizer of human intestinal tract, has been increasingly implicated in several infective diseases including endocarditis, prosthetic valve endocarditis, and late-onset sepsis in very-low birth-weight neonates. *S. capitis* has biofilm production capacity especially on medical placement devices, which contributes to its capacity to invade surgical disinfectants (*10*). *S. haemolyticus* is another pathogenic CoNS. Genomic plasticity accounting for several insertion sequences and plasmids some of which confer multiple antibiotics resistance, notably against beta-lactams and glycopeptides in these strains, has been suggested (*11,12*). As a nosocomial pathogen, *S. haemolyticus* is primarily a blood culture isolate with capacity to invade immune defenses of compromised individuals. However, data on CoNS isolation from non-hospital environment and genetic resistance mechanisms are sparsely available. In this study, we characterized the molecular mechanisms of multiple antimicrobial resistant *S. capitis* and *S. haemolyticus* strains isolated from nature and experimentally challenged the possibility of horizontal gene transmission to *S. aureus*.

2. Materials and Methods

2.1. *Isolation of strains and determination of minimum inhibitory concentration*

Colonies were isolated from the environment using mannitol-salt agar medium plates supplemented with tetracycline; 2 µg/mL. Minimum inhibitory concentration (MIC) was determined according to the following procedure. Isolated single colony was cultured overnight in tryptic soy broth medium overnight. Full growth was diluted with sterilized physiological saline to give optical density at 600 nm (Shimadzu UV-1280 spectrophotometer), 0.5 and re-diluted (1/150). Re-diluted culture (100 µL) was served with the antibiotics (100 µL) into 96-well plate, two-fold decimally diluted and incubated at 37°C. Cation-adjusted Mueller Hinton broth (CA-MHB) supplemented with calcium (25 mg/mL) and magnesium (12.5 mg/mL) ions were employed for all dilutions. MIC was defined as the lowest concentration of antimicrobial agent that inhibits growth of the organism in the micro-dilution wells as detected by the unaided eye, using the 96-well micro-titer plate format.

2.2. *Genomic DNA isolation, whole genome sequencing and assembly*

Total genomic DNA from 5 mL full growth was isolated using DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following manufacturer's recommendation. The integrity and purity of the DNA was confirmed by agarose gel electrophoresis and NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). Whole genome sequencing was performed using 100 ng of the total DNA as quantified by Qubit 3.0 fluorometer (ThermoFisher) as described previously (*13*). Briefly, the barcoded library of 400 base-reads was prepared using the Ion Xpress™ Plus Fragment Library Kit (ThermoFisher). The quality, quantity, and size distribution of the libraries were determined using an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, United States). The libraries were then enriched in an Ion 318™ Chip v2 using Ion Chef (ThermoFisher), and subsequent sequencing was performed in the Ion PGM System (ThermoFisher). The reads were then assembled using SPAdes 3.11 (*14*) and further analysis of the assembly was performed using CLC Genomics Workbench ver 11.0 (Qiagen Bioinformatics, Aarhus, Denmark).

2.3. *Conjugation assay*

S. aureus (RN4220 strain) selected with fusidic acid (6.3 µg/mL) and rifampicin (1 µg/mL) was employed as recipients. One mL full growth (about 10^9 CFU each) of donors (*S. capitis*, *S. haemolyticus*) and recipients were mixed on ice, and centrifuged (5,000 rpm, 4°C, 5 min) and dried on 0.22-µm membrane filter (Millipore Sigma, MA-USA). Donors and recipient control experiments were also included. Conjugation was performed on Brain Heart Infusion-agar (1.5%) overnight at 37°C. Cells were recovered in sterilized saline and transconjugants were selected on BHI-agar supplemented with fusidic acid (6.3 µg/mL), rifampicin (1 µg/mL) and tetracycline (1 µg/mL). Incubation at 37°C was performed for 48 hs.

2.4. *Statistical analysis*

Appearance ratio of transconjugants was compared using Graph Pad Prism 5. $*p < 0.01$ was considered statistically significant by unpaired student's *t*-test with 99% confidence interval.

2.5. *Data availability*

This whole-genome project including the assembled contigs and raw reads has been deposited at NCBI Bio Project under the accession PRJNA471195. The DDBJ/ENA/GenBank accession numbers of each assemblies are: RIYT00000000, RIYU00000000, and RIYV00000000 for Tc^R-3, Tc^R-5, and Tc^R-7, respectively.

3. Results

3.1. *Antibiotic susceptibility profiling of isolates*

Table 1. Identification of isolates

Strain	Closest homolog	% identity, gap
Tc^R-3	*Staphylococcus capitis* strain AYP1020	1554/1554 (100%), 0
Tc^R-5	*Staphylococcus haemolyticus* JCSC1435	1554/1554 (100%), 0
Tc^R-7	*Staphylococcus haemolyticus* JCSC1435	1554/1554 (100%), 0

To characterize the isolates, 16S rDNA of whole genome analyzed isolates were extracted and blasted in National Center for Biotechnological Information (NCBI) database.

We focused on the isolation and molecular characterization of environmentally mobilized staphylococci bacteria with antibiotic resistant phenotype. Using tetracycline supplemented mannitol-salt agar medium, we isolated antibiotic resistant bacteria colonies. Cells having mannitol utilization capacity were isolated. Single colony isolation was performed using Brain Heart Infusion-agar plate. To identify the bacteria, we extracted the 16S rDNA of the sequenced isolates from the whole genome data. BLAST algorithm was performed in NCBI database. Result suggested that analyzed strains were *S. capitis* and *S. haemolyticus*, with 100% similarity to available literature data (Table 1). Antimicrobial susceptibility assay of these isolates via minimum inhibitory concentration (MIC) determination was determined. Antibiogramic data (Table 2) showed that the isolates displayed tetracycline resistance. Further examination suggested that the environmental bacteria also displayed decreased sensitivity (elevated MICs) against mechanistically diverse antimicrobial agents compared to CLSI standard breakpoints (*15*) including norfloxacin, kanamycin, and erythromycin with no observed decreased susceptibility against rifampicin, chloramphenicol and vancomycin. These findings indicate that the isolates possess genetic mechanisms that allow for its existence even in the face of environmental stress.

Table 2. Antibiogram of isolated environmental bacteria

Antibiotic	MIC (μg/mL)		
	Tc^R-3	Tc^R-5	Tc^R-7
Tetracycline	128	32	32
Kanamycin	128	1	1
Norfloxacin	1	128	128
Erythromycin	0.5	32	32
Teicoplanin	0.5	4	4
Ampicillin	1	8	8
Cefcapene	1	1	1
Cefditoren	0.5	0.5	0.5
Oxacillin	0.25	0.25	0.25
Chloramphenicol	8	4	4
Rifampicin	< 0.008	< 0.008	< 0.008
Vancomycin	2	1	1

Table 3. General feature(s) and genome statistics of the assembled sequences

Feature of chromosome	Value for strain		
	Tc^R-3	Tc^R-5	Tc^R-7
Length of sequence (bp)	2,515,208	2,563,958	2,542,447
G+C content (bp)	824,989	835,851	828,838
G+C contents (%)	32.8	32.6	32.6
Number of reads	1,476,795	603,500	643,974
Coverage	160	66	73
N50 (bp)	466,109	94,107	79,590
L50 (No. of contigs)	2	12	9

3.2. *Diversity of antimicrobial resistant genes in the isolates*

To provide genetic evidence and elucidate the molecular mechanisms behind the multidrug resistance of the isolates, we intended to identify the resistant genes in the isolates by whole genome sequencing. Genomic DNA of representative multi-drug resistant isolates (Tc^R-3, Tc^R-5 and Tc^R-7) were extracted, sequenced and assembled. Sequence data assembly revealed a genome size range of 2.5 M bp. G + C content was approximately 33%. Reads (1.48×10^6, 6.03×10^5 and 6.44×10^5) and genome coverages (160, 66, and 73) for Tc^R-3, Tc^R-5, and Tc^R-7 respectively were obtained (Table 3). Genome annotation revealed the presence of antibiotic resistance genes: *aac*(6')-*aph*(2'), *blaZ*, *mph*(C), *msr*(A), *vga*(A) and *Tet*(K) (Table 4).

3.3. *Roles of coagulate negative Staphylococci in spread of antibiotic resistance*

To understand the role of these isolates in spreading of antibiotic resistance, conjugation assay was performed using *S. aureus* RN4220, a laboratory strain, harboring fusidic acid and rifampicin resistances as recipients. Assay was performed as described in methodology section. Results revealed that the colony-forming units (CFUs) of *S. aureus* RN4220 cells that appeared on the selection plate after conjugation increased and significantly differed from each recipient controls (Figure 1). Donor controls yielded no colonies on selection plate.

4. Discussion

Notwithstanding calls for better picture on the reservoir of genes in our surrounding and how they aid acquisition of resistance phenotypes in clinically relevant pathogens; available genetic information are sparse on the resistome of environmental bacteria. In that regard, this study

Table 4. Drug resistant genes identified in the assembled genomes

Strain	Closest bacteria homolog	Identified gene	Gene function
Tc^R-3	*S. capitis*	*aac(6')-aph(2')*	Aminoglycoside resistance
		Tet (K)	Tetracycline resistance
Tc^R-5	*S. haemolyticus*	*blaZ*	Beta-lactam resistance
		mph(C)	Macrolide resistance
		msr(A)	Macrolide, Lincosamide and Streptogramin B resistance
		vga(A)	Streptogramin B resistance
		tet(K)	Tetracycline resistance
Tc^R-7	*S. haemolyticus*	*blaZ*	Beta-lactam resistance
		mph(C)	Macrolide resistance
		msr(A)	Macrolide, Lincosamide and Streptogramin B resistance
		vga(A)	Streptogramin B resistance
		tet(K)	Tetracycline resistance

The nucleotide sequences of the assembled genomes were analyzed in the CLC Genomics Workbench against the database of Find Resistance using default parameters.

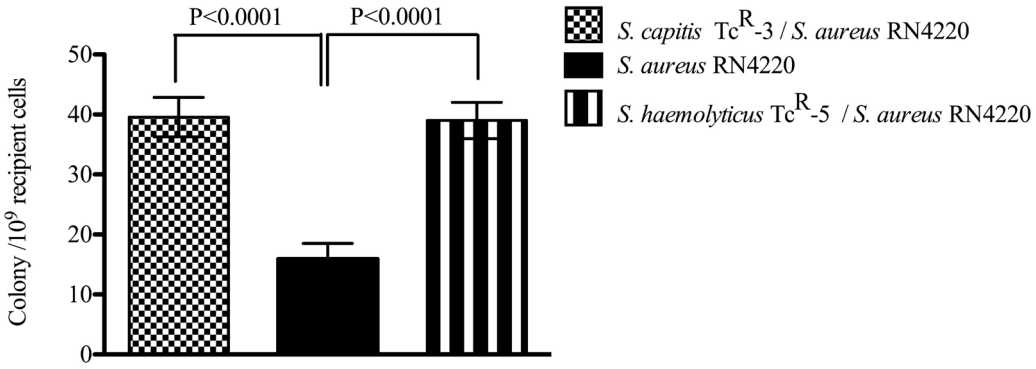

Figure 1. Conjugational transduction of tetracycline resistance to *S. aureus*. Conjugation assay was performed on BHI-agar media. To determine total number of recipient present after conjugation, recovered suspensions were diluted and spread on selective media plate and ratio of transconjugants to total recipient cells determined after 48hrs. Experiments were repeated thrice in triplicates. Plots show mean and standard deviation. Statically analysis was performed by unpaired Student's *t*-test with 99% confidence interval.

investigated multiple antimicrobials resistant bacteria isolated from a non-clinical environment in Japan. In addition to clinically relevant antimicrobial agents, these strains can transport environmental biocides and disinfectants which may allow persistence in the environment and subsequent transmission on to a suitable host. Molecular analysis of three isolates from the library showed the presence of multiple antibiotic resistant genes: *aac*(6')-*aph*(2'), *bla*Z, *mph*(C), *msr*(A), and *Tet*(K) suggetsing the origin of the MDR phenotype. Decreased sensitivity to tetracycline in staphylococci mediated by expression of a ribosomal protection protein molecule encoded by *Tet*(K) or *Tet*(M) genes, or via efflux mechanisms has been investigated (*17*). Aminoglycoside-modifying enzyme gene, *aac*(6′)-*aph*, is the most common antimicrobial resistant gene conferring aminoglycoside, including kanamycin resistance, in staphylococci. The capacities of the *blaZ* and *msr*(A) genes in conferring beta-lactams and macrolide resistances respectively are well-known. In particular, *msr*(A) gene encode ATP-dependent efflux pump conferring resistance to 14- and 15-membered macrolides, including erythromycin. Still, none of the identified genes is associated with decreased sensitivity to norfloxacin and teicoplanin. The *mph*(C) and *msr*(A) are key candidates genes, though none is previously associated with efflux of fluoroquinolones or glycopeptides. Further studies are required to elucidate the actual resistance mechanisms. This data supports the need for the controlled use of antibiotics and establishes the criticality of CoNS as a reservoir of antibiotic resistant determinants. In the knowledge of the authors, this is the first study to document the isolation of *S. capitis* harboring multiple antibiotic resistant genes from non-clinical (non-hospital) environment. Despite untiring efforts towards analyses of resistant clinical bacterial isolates, the exact process of the acquisition of resistance to these antimicrobials remains unclear. For environmental microbial resistant armamentarium, the stories are not different and further studies are thus necessitated. Understanding these mechanisms could potentially aid antimicrobial agents development. Presence of plethora of genes responsible for antibiotic resistance suggests

that strains *S. haemolyticus* and *S. capitis* could present a potential threat to human health. Environmentally mobilized MDR bacteria such *S. haemolyticus* and *S. capitis* might serve as a foothold towards transference of AMR conferring foreign genetic elements into other clinically relevant pathogens.

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Conflict of Interest

K.S. is a consultant for Genome Pharmaceuticals Institute Co., Ltd.

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Original Article

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Generic selection criteria for safety and patient benefit [VIII]: Comparing the physicochemical and pharmaceutical properties of brand-name and generic diclofenac sodium tapes

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Summary **With respect to diclofenac sodium-containing tape preparations of nonsteroidal antiphlogistic drug, we compared the pharmaceutical properties (pH, elongatedness, water-vapor permeability, adhesive force, and peeling-force) of 11 medicinal drugs (2 brand-name and 9 generic drugs) to obtain evidence for product selection in line with the needs of the patient. The elongatedness of the generic drugs Teikoku (1.39), Yutoku (1.40), and Nippon-zoki (1.43) were significantly higher than the brand-name drug Voltaren® (1.22). The adhesive force was measured using the probe tack test and the inclined ball tack test. The probe tack test results of Naboal® (6.8 N/cm^2), Teikoku (6.1 N/cm^2), Yutoku (5.9 N/cm^2), Nippon-zoki (6.2 N/cm^2), and Rakool (6.2 N/cm^2) were higher than that of Voltaren (2.0 N/cm^2). The inclined ball tack test results of Naboal (18.0), Teikoku (24.0), Yutoku (21.5), and Nippon-zoki (22.7) were also higher than that of Voltaren (7.2). Concerning peeling-force measurement, the 90° peeling-forces of Naboal (0.95 N), Teikoku (0.96 N), Yutoku (0.94 N), and Nippon-zoki (1.01 N) were higher than that of Voltaren (0.68 N). These results show that there were marked differences in the feeling of use of each product between the brand-name and generic drugs. The pharmacist indicates the basis for selection of a preparation according to the feeling of use desired by each patient. It has become possible to recommend products suitable for each patient, which will allow pharmacists to provide products according to the needs of each patient when a brand-name drug is changed to a generic one.**

Keywords: Transdermal therapeutic drug, brand-name drug, generic drug, diclofenac sodium tape

1. Introduction

It is estimated that the medical care expense for citizens in Japan will be 52.3 trillion yen in 2025 from 34.8 trillion yen in 2008, among which the medical expenses of the late-stage medical care system for the elderly will increase from 11.4 trillion yen to 24.1 trillion yen (*1*). As a solution to this problem, the use of generic drugs containing the same principal ingredients as brand-name drugs is recommended. However, the brand-name and generic drugs differ in the manufacturing processes and additives (*2*), and their use has not progressed as a result of external appearance, feeling of use, and safety being not the same as brand-name drugs. Especially, the patient finds it easy to feel a difference in the feeling of use of a patch, and many cases have been reported cases in which patients complain of differences in the feeling of use when a brand-name drug is changed to a generic drug (*3*).

Diclofenac sodium tape is a nonsteroidal anti-inflammatory drugs (NSAIDs) discovered in 1965, and it is widely known that it shows strong anti-inflammatory and analgesic effects. It has gained a high reputation for many years. In Japan, "Voltaren® tape (*4*)"

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and "Naboal® tape" as patches were launched in 2004 as brand-name drugs.

In this study, we conducted a comparative evaluation of the brand-name and generic drugs of diclofenac sodium tape, and conducted research aimed at providing information that could be the basis for drug selection at the pharmacy. In addition, among the two types of the brand-name drugs, "Voltaren® tape" with many clinical research reports was compared with other patches (*5*).

2. Materials and Methods

2.1. *Materials*

As diclofenac sodium tape (7 cm × 10 cm), brand-name drugs, Voltaren® tape 15 mg (Dojin Iyaku-kako Co., Ltd., Tokyo, Japan) and Naboal® tape 15 mg (Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan), and generic drugs, diclofenac sodium tape 15 mg "Teikoku" (Teikoku Seiyaku Co., Ltd., Kagawa, Japan), diclofenac sodium tape 15 mg "Yutoku" (Yutoku Pharmaceutical Ind. Co., Ltd., Saga, Japan), diclofenac sodium tape 15 mg "Sanwa" (Sanwa Kagaku Kenkyusho Co., Ltd., Aichi, Japan), diclofenac sodium tape 15 mg "NP" (Nipro Pharma Co., Ltd., Osaka, Japan), diclofenac sodium tape 15 mg "JG" (Nihon Generic Co., Inc., Tokyo, Japan), diclofenac sodium tape 15 mg "Nippon-zoki" (Nippon Zoki Pharmaceutical Co., Ltd., Osaka, Japan), diclofenac sodium tape 15 mg "Nichi-Iko" (Nichi-Iko Pharmaceutical Co., Ltd., Toyama, Japan), diclofenac sodium tape 15 mg "Towa" (Towa Pharmaceutical Co., Ltd., Osaka, Japan), and diclofenac sodium tape 15 mg "Rakool", (Mitomo Yakuhin Co., Ltd., Tokyo, Japan), were purchased and used in this experiment (Table 1). All the other reagents were of analytical grade.

Table 1 shows the product name, abbreviated name, classification, company name, and lot number of the diclofenac sodium tape used in this study. Lot numbers A and B were set according to the time when the test was carried out.

2.2. *Measurement of pH*

We measured pH values, as described by Wada *et al*. (*6,7*). Briefly, each preparation was cut into sections measuring 20 × 30 mm, placed in sample bottles containing 20 mL of purified water, and agitated for 185 rpm × 24 h. Subsequently, the pH of the solution was measured using a Benchtop pH meter F-74 and ISFET pH electrode 0040-10 D (HORIBA, Ltd., Kyoto, Japan). For each product, measurement was conducted 6 times at 24 ± 2°C, and the mean was adopted as its pH value.

2.3. *Measurement of the elongatedness*

An elongatedness test was performed, as described by Wada *et al*. (*7*). The end (70 mm) of a section of each product measuring 70 × 100 mm was fixed on an experimental table with the adhesive surface. A weight (300 g) was suspended on the other short side, and the length (mm) of the preparation after 10 seconds was measured. The elongatedness was calculated from the values before and after. For each product, a measurement was conducted 6 times, and the mean was regarded as the elongatedness. The elongatedness of change in elongation was calculated by the following formula: elongatedness (E) = L/L_0 [L: Length (mm) after 10 seconds, L_0: Length before test (mm)]

2.4. *Measurement of water-vapor permeability*

The water-vapor permeability test was performed, as described by Sugino *et al*. (*8*). Briefly, 10 mL of purified water was placed in an Erlenmeyer flask, and its opening was covered with a round section of each product measuring 20 mm in diameter. After the weight was measured, each sample was allowed to stand for 24 hours in an environment chamber KCL-2000W (Tokyo Rikakikai Co., Ltd., Tokyo, Japan) under the following conditions: temperature, 25°C; relative humidity, 55%. Additionally, the weight was measured. As a control, the weight of the Erlenmeyer flask containing purified water

Table 1. Tape products used in this experiment

Product name	Abbreviated name	Class	Company name	Lot number A	Lot number B
Voltaren® Tape 15 mg	Voltaren	BN	Dojin Iyaku-kako Co., Ltd.	40420	170190
Naboal® Tape 15 mg	Naboal	BN	Hisamitsu Pharmaceutical Co., Inc.	50103	U201U
Diclofenac sodium tape 15 mg "Teikoku"	Teikoku	GE	Teikoku Seiyaku Co., Ltd.	4J150	7H010
Diclofenac sodium tape 15 mg "Yutoku"	Yutoku	GE	Yutoku Pharmaceutical Ind. Co., Ltd.	5C050	8B040
Diclofenac sodium tape 15 mg "Sanwa"	Sanwa	GE	Sanwa Kagaku Kenkyusho Co., Ltd.	AH00601	AL00101
Diclofenac sodium tape 15 mg "NP"	NP	GE	Nipro Pharma Co., Ltd.	LM068	17R201
Diclofenac sodium tape 15 mg "JG"	JG	GE	Nihon Generic Co., Ltd.	407060	710170
Diclofenac Na tape 15 mg "Nippon-zoki"	Nippon-zoki	GE	Nippon-zoki Pharmaceutical Co., Ltd.	4J130	7H040
Diclofenac Na tape 15 mg "Nichi-Iko"	Nichi-Iko	GE	Nichi-Iko Pharm. Co., Ltd.	IP0801	KD2101
Diclofenac Na tape 15 mg "Towa"	Towa	GE	Towa Pharmaceutical Co., Ltd.	A102	A0122
Diclofenac Na tape 15 mg "Rakool"	Rakool	GE	Mitomo Yakuhin Co., Ltd.	G20XR	A02XW

BN: brand-name drug, GE: generic drug.

(10 mL) not covering the opening was measured. The water-vapor permeability was calculated from the rate of change in the weight using the following formula: water-vapor permeability (%) = $(W_0 - W_1/Ww_0 - Ww_1) \times 100$ [W_0: weight before testing (g), W_1: weight after testing (g), Ww_0: weight of control before testing (g), Ww_1: weight of control after testing (g)]. With respect to each product, measurement was conducted 6 times, and the mean was regarded as the water-vapor permeability (%).

2.5. *Measurement of adhesion testing*

2.5.1. *Probe tack testing method*

The adhesive-force was measured according to the probe tack testing method established in the Japanese Pharmacopoeia 17th Edition (*9*). After sticking a tape preparation to the jig of the probe tack examination device MED-IS-20N (Imada Co., Ltd., Aichi, Japan), without looseness, and placing the specified columnar probe in contact with the adhesive surface of the tape for a fixed time, the probe was removed vertically from the adhesive surface at a speed of 5 mm/s. Then, the maximum load required for peeling was determined.

2.5.2. *Inclined ball tack testing method*

The adhesive-force was measured according to the inclined ball tack testing method established in the Japanese Pharmacopoeia 17th Edition (*9*). A section of each product was attached to the inclined ball tuck examination device TransTack® Ball-Tack Meter Trans Tack-W (CosMED Pharmaceutical Co. Ltd., Kyoto, Japan). The maximum ball number (No. 1-32) was measured by the inclined ball tuck examination device. With respect to each product, measurement was conducted 6 times, and the mean was regarded as the adhesive force.

2.6. *Measurement of the peeling-force*

2.6.1. *90° or 180° peel test*

The peeling-force was measured according to the Japanese Pharmacopoeia 17th Edition (*9*). Briefly, a slide table P90-200N (Imada Co., Ltd., Aichi, Japan) for 90° or 180° peeling test was fixed on an MX2-500N (Imada Co., Ltd., Aichi, Japan) stand for measurements according to the method of Wada *et al*. (*7*). On its surface, a section of each product (90°: 35 mm × 65 mm, tong hold 5 mm; 180°: 35 mm × 54 mm, tong hold 30 mm) was longitudinally attached. In addition, a crimp roller (2 kg: Japanese Industrial Standards: JIS, Z0237: 2009) was rolled over each drug. Subsequently, the peeling-force was measured by pinching a 5 or 30 mm area of the upper margin with a film clip FC-40 (Imada Co., Ltd., Aichi, Japan) and pulling it at a constant rate (5 mm/sec) so that the adhesive surface was peeled off to be 90° or 180° to a digital force gauge, ZP-20N (Imada Co., Ltd., Aichi, Japan), until the tape had been completely exfoliated from the stainless plate. With respect to each product, measurement was conducted 6 times, and the mean was regarded as the peeling-force.

2.6.2. *90° peel test using EVA (Ethylene-vinyl acetate) membrane*

The peeling-force was measured according to the Japanese Pharmacopoeia 17th Edition (9). According to the method of 2.6.1, after attaching the EVA membrane to the stainless plate, each product was further stuck thereon, and was performed 6 times for each patch. Incidentally, the peel test was measured 6 times for each patch 24 hours after attaching (24 h) and 24 hours after attachment in tepid water (40°C) for 30 minutes (24 h + tepid water).

2.7. *Statistical analysis*

For each experimental result, the values were compared using Dunnett's test, Tukey-Kramer-test and Bonferroni/Dunn-test of the multiple comparison test or Pearson's correlation coefficient test (*10*). A *p*-value of 0.05 (marked with *) or 0.01 (marked with **) was regarded as significant in the figure.

3. Results

3.1. *Measurement of pH*

The pH is an important factor reflecting the stability of the active component in each preparation or skin irritability. The results of pH measurements for each product are shown in Figure 1. There were marked differences in pH among the brand-name and generic products. The pH values of a brand-name drug Naboal (pH 5.2), generic drugs Sanwa (pH 5.2), NP (pH 5.1),

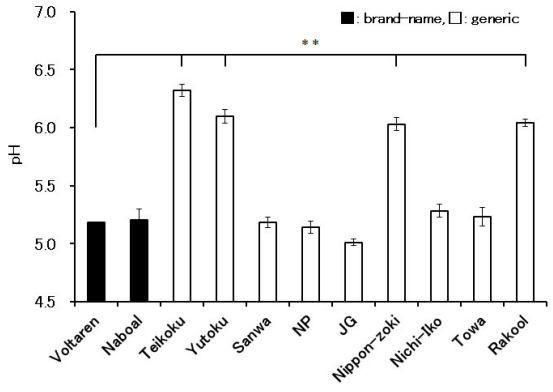

Figure 1. pH measurement of various preparations (Lot #A) (*n* = 6). **$p < 0.01$ (*vs*. Voltaren, Dunnett-test).

JG (pH 5.0), Nichi-Iko (pH 5.3) and Towa (pH 5.2) were similar to that of a brand-name drug Voltaren (pH 5.2). On the other hand, the pH values of the generic drugs Teikoku (pH 6.3), Yutoku (pH 6.1), Nippon-zoki (pH 6.0) and Rakool (pH 6.0) were significantly higher than the brand-name drug Voltaren (pH 5.2). Significance tests of these drugs were conducted. There were significant differences between Teikoku, Yutoku, Nippon-zoki, and Rakool ($p < 0.01$) (Figure 1).

3.2. *Measurement of elongatedness*

In many products, a stretchy material is used for the support layer to prevent turning-up or peeling after attachment to articular regions such as the knees and elbows. The results of elongatedness measurement are shown in Figure 2. The elongatedness of the generic drugs Teikoku (1.39), Yutoku (1.40), and Nippon-zoki (1.43) were significantly higher than the brand-name drug Voltaren (1.2). On the other hand, the elongatedness of a brand-name drug Naboal (1.22), generic drugs Sanwa (1.20), NP (1.20), JG (1.23), Nichi-Iko (1.19), Towa (1.24) and Rakool (1.26) were similar to that of a brand-name drug Voltaren (1.22). In addition, there were significant differences between Teikoku, Yutoku, Nippon-zoki, and Rakool ($p < 0.01$) (Figure 2).

3.3. *Measurement of water-vapor permeability*

The water-vapor permeability of each preparation may induce maceration stimuli when the skin water permeability is low on attachment. We measured the water-vapor permeability of each product. The results are shown in Figure 3. There were marked differences in the water-vapor permeability among the products; the water-vapor permeabilities of a brand-name drug Naboal (1.63%), generic drugs Sanwa (1.74%), NP (1.61%), JG (1.65%), Nichi-Iko (2.45%) and Towa (1.82%) were higher than the brand-name drug Voltaren (0.92%). On the other hand, Yutoku (0.42%), Nippon-zoki (0.67%) and Rakool (0.39%) were lower than that of Voltaren (0.92%).

3.4. *Measurement of adhesive force*

3.4.1. *Probe tack testing*

Preparations with a strong adhesive force may not peel off when attached to the skin, whereas those with a weak adhesive force tend to peel off. We measured the adhesive force of each product. The results (bar graph) of the probe tack testing of each product are shown in Figure 4. There were marked differences in the adhesive force among the products; the adhesive forces of the brand-name drug Naboal (6.8 N/cm^2) and the generic drugs Teikoku (6.1 N/cm^2), Yutoku (5.9 N/cm^2), Nippon-zoki (6.2 N/cm^2), and Rakool (6.2 N/cm^2) were higher than that of Voltaren (2.0 N/cm^2). In addition, the results showed significant differences between all products and Voltaren ($p < 0.01$) (Figure 4).

3.4.2. *Inclined ball tack testing*

The results (line graph) of the inclined ball tack testing

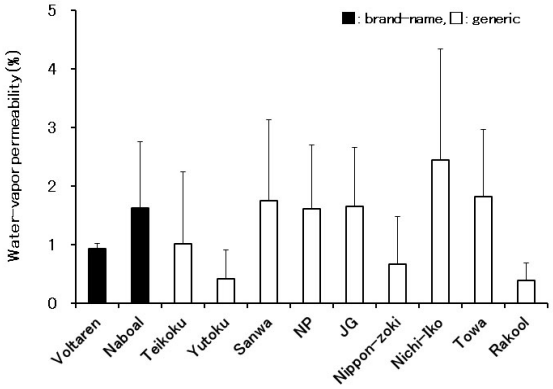

Figure 3. Water-vapor permeability of various preparations (Lot #A) ($n = 6$).

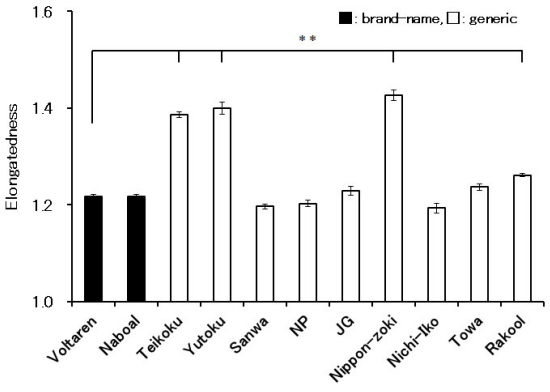

Figure 2. Elongatedness of various preparations (Lot #A) ($n = 6$). **$p < 0.01$ (*vs*. Voltaren, Dunnett-test).

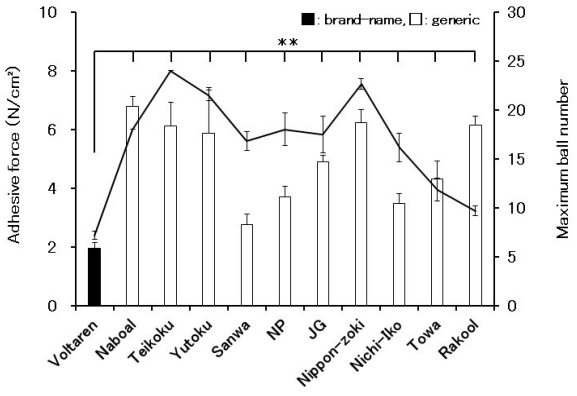

Figure 4. Adhesive force of various preparations (Lot #B) (n = 6). Probe tack testing (bar graph), Inclined ball tack testing (line graph) **$p < 0.01$ (*vs*. Voltaren, Dunnett-test).

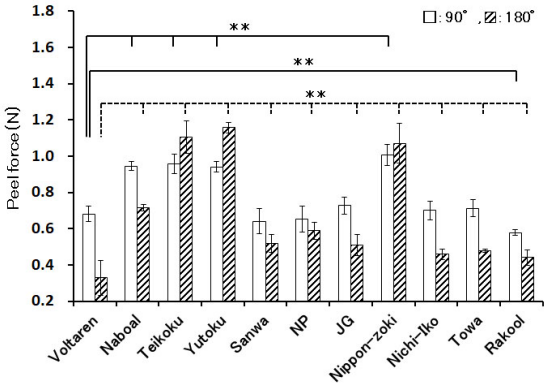

Figure 5. Comparison of 90° and 180° peel force in various preparations (Lot #B) (*n* = 6). $^{**}p < 0.01$ (*vs*. Voltaren, Dunnett-test, solid line: 90°, dotted line: 180°).

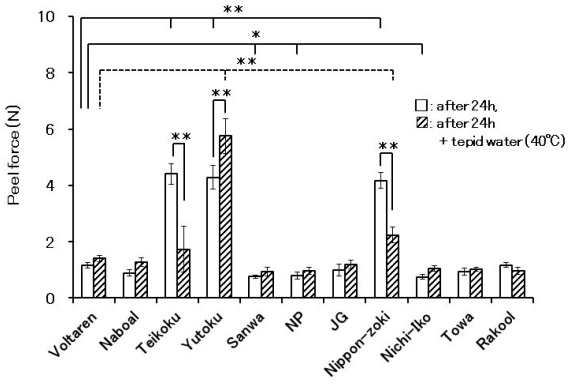

Figure 6. Comparison of 90° peel force in various preparations (Lot #A) (*n* = 6). $^{*}p < 0.05$, $^{**}p < 0.01$ (*vs*. Voltaren, Dunnett-test; Teikoku *vs*. Teikoku + tepid water (40°C), Yutoku *vs*. Yutoku + tepid water, Nippon-zoki *vs*. Nippon-zoki + tepid water: Tukey-Kramer-test and Bonferroni/ Dunn-test; solid line: after 24 h, dotted line: after 24 h + tepid water).

of each product are shown in Figure 4. There were marked differences in the adhesive force among the products; the adhesive forces of the brand-name drug Naboal (18.0) and the generic drugs Teikoku (24.0), Yutoku (21.5), and Nippon-zoki (22.7) were higher than that of Voltaren (7.2). The numbers in parentheses for each of the above products indicate average ball number value. In addition, the results showed significant differences between all products ($p < 0.01$) (Figure 4).

3.5. *Measurement of peel force*

3.5.1. *Measurement of 90° and 180° peel force*

The peeling-force refers to a force required to peel off the preparation after attachment. We measured the 90° and 180° peeling-force of each product. The results are shown in Figure 5. The 90° peeling-forces of Naboal (0.95 N), Teikoku (0.96 N), Yutoku (0.94 N), and Nippon-zoki (1.01 N) were higher than that of Voltaren (0.68 N), whereas those of Rakool (0.58 N) was lower than that of Voltaren. Similarly, the 180° peeling-forces of Naboal (0.72 N), Teikoku (1.11 N), Yutoku (1.16 N), and Nippon-zoki (1.07 N) were higher than that of Voltaren (0.33 N). In addition, significance tests of various preparations were conducted. The brand-name drug Naboal and generic drugs Teikoku, Yutoku, and Nippon-zoki showed significant differences in comparison with Voltaren ($p < 0.01$) (Figure 5, 90° peel force). All products showed significant differences in comparison with Voltaren ($p < 0.01$) (Figure 5, 180° peel force).

3.5.2. *Measurement of 90° peel force using EVA membrane*

The results of the 90° peel force using the EVA membrane of each product are shown in Figure 6. The peeling-forces of Teikoku (4.41 N), Yutoku (4.29 N), and Nippon-zoki (4.18 N) were higher than that of Voltaren (1.15 N). Teikoku, Yutoku, and Nippon-zoki showed significant differences in comparison with Voltaren ($p < 0.01$). Similarly, the peeling-forces after 30 minutes in tepid water (40°C) of Yutoku (5.76 N) and Nippon-zoki (2.24 N) were higher than that of Voltaren (1.40 N). Yutoku and Nippon-zoki showed significant differences in comparison with Voltaren ($p < 0.01$) (Figure 6).

In addition, in the case of 24 hours and 24 hours + tepid water (40°C), when compared with the values of Teikoku, Yutoku, and Nippon-zoki, the values of Yutoku increased to 1.47 N, but Teikoku and Nippon-zoki decreased to 2.68 N and 1.94 N, respectively. There were significant differences between Teikoku, Yutoku, and Nippon-zoki ($p < 0.01$) (Figure 6).

4. Discussion

NSAIDs are widely used for the treatment of various disorders in expectation of analgesic/ anti-inflammatory actions, but the incidence of adverse reactions has been reported to be higher in tape preparations compared with other dosage forms (*11*). Contact dermatitis is the most frequent adverse reaction, accounting for 80% of all adverse reactions. In elderly patients, who have thinner epidermis and are more sensitive to stimuli than younger patients, the incidence of adverse reactions is particularly high, and special attention to adverse reactions is required in using tape preparations.

Usually, the corneum is mildly acidic at a pH of 4.5 - 6.5 (*12*), and the use of a preparation with a pH that markedly deviates from this range may well induce skin irritation or skin trouble. As shown in Figure 1, the pH of all products was 5.0 - 6.3, and irritation of skin due to the pH is considered to be mild.

Elongatedness is considered to affect the feeling of use of adhesive skin patches when they are applied to flexible parts such as the knee and arm. As shown in Figure 2, Teikoku (1.39), Yutoku (1.40), and Nippon-

zoki (1.43), which showed significantly higher elongation rates than the brand-name drug Voltaren (1.22), were 14.0-17.2% more stretchable and are considered to be suited for application at movable areas, such as the joints, and to cause wide differences in the feeling of use in flexible areas.

Next, products with low water-vapor permeability have been reported to increase the wetness of the skin at the site of application, reduce the strength of attachment of corneocytes, and promote cuticle exfoliation (*13*). As shown in Figure 3, the water-vapor permeability (%) calculated from the amount of water-vapor permeation during 24 hours after application of each product was significantly higher in Nichi-Iko (2.45%) than in the brand-name drug Voltaren (0.92%), and Nichi-Iko is considered to be more permeable to moisture on the skin and less likely to cause skin wetting. Products with low water-vapor permeability are considered to allow less water to permeate and to be more likely to cause skin wetting, and Yutoku (0.42%) and Rakool (0.39%) were shown to be more likely to wet the application site than the brand-name drug Voltaren.

Next, tape preparations, which are attached to the skin, must have appropriate adhesive force. Therefore, we performed probe tack testing to compare the adhesive force of various products in peeling off. As shown in Figure 4, the adhesive force was significantly higher in Naboal (6.8 N/cm^2), Teikoku (6.1 N/cm^2), Yutoku (5.9 N/cm^2), Nippon-zoki (6.2 N/cm^2), and Rakool (6.2 N/cm^2) compared with Voltaren (2.0 N/cm^2), and these products are considered to be more adhesive. Similarly, from the results of the inclined ball tack testing, the adhesive force is considered to be stronger in the products with a higher ball number than the brand-name drug Voltaren (mean ball number: 7.2). Also, since the initial adhesiveness, which is derived from the adhesive components of the tape preparation (*e.g.*, styrene-isoprene-styrene block copolymer), can be evaluated by the inclined ball tack testing (*8*), we consider that Naboal (18.0), Teikoku (24.0), Yutoku (21.5), NP (18.0), JG (17.5), and Nippon-zoki (22.7), which showed mean ball numbers higher than that of Voltaren, have a stronger initial adhesive force than Voltaren. We further examined the correlation between probe tack testing and inclined ball tack testing using Pearson's correlation coefficient and found a significant difference (p = 0.0498) positive correlation (r = 0.52) between the two tests. Thus, following the initial adhesion of the tape product, the adhesiveness of products to the skin is considered to depend on adhesion between the skin and the product and cohesive force of the product itself that resists peeling off (*14*), and these two factors are considered to determine the total adhesive force, but the positive correlation demonstrated between the probe tack and inclined ball tack testing results is considered to suggest that the initial adhesive force is nearly proportionate to the cohesive force of the product itself that resists peeling off.

Next, unlike oral or injection preparations, tape products must be removed from the skin after a period of application. Since the force required to peel off the product greatly affects the compliance, the peeling force is an important criterion in its evaluation. The peeling force of a product is considered to depend on the adhesive force and cohesive force of the adhesive agent used on the adhesive surface of the product. Figure 5 compares the peeling forces in removing various tape products at 90° and 180°. First, at 90°, the peeling force was significantly higher in Naboal (0.95 N), Teikoku (0.96 N), Yutoku (0.94 N), and Nippon-zoki (1.01 N) than in the brand-name drug Voltaren (0.68 N). At 180°, the peeling force was significantly higher in Naboal (0.72 N), Teikoku (1.11 N), Yutoku (1.16 N), and Nippon-zoki (1.07 N) than in the brand-name drug Voltaren (0.33 N). In addition, when the correlation between the results of the peeling force testing were compared between 90° and 180° using Pearson's correlation coefficient, a high positive correlation (r = 0.88) with statistical significance (p = 0.0002) was observed between 90° and 180°. Moreover, when the peeling force was compared between 90° and 180°, it was 0.68 N and 0.33 N, respectively, in Voltaren, indicating that it can be removed with about half the force at 180°. Similar results were obtained with Naboal, Sanwa, NP, JG, Nichi-Iko, Towa, and Rakool, but Teikoku, Yutaka, and Nippon-zoki could be removed with a weaker force at 90° than at 180°. From these results, the angle is a very important factor in peeling off tape products. Although we measured the peeling force only at 90° and 180° in this study, the stimulus to the skin is considered to be mitigated by appropriately adjusting the angle of removal in each product.

Next, regarding the dose and administration method, since the package inserts of the products used in this study say, "apply to the affected area once a day", we attached each product to an EVA membrane (*15*), which is used as an artificial permeable membrane, with a fixed load and compared the peeling force in removing it from the EVA membrane after 24 hours. As shown in Figure 6, the peeling force was significantly higher in Teikoku (4.41 N), Yutoku (4.29 N), and Nippon-zoki (4.18 N) than in Voltaren (1.15 N). Therefore, if Voltaren (1.15 N) or Naboal (0.89 N) has been changed to one of the above generic drugs because of the insufficient adherence of the brand-name drug, it is more likely to remain attached, but the patient may feel pain in the skin in removing it because of the large force, 3.63-3.83 times greater, required to peel it off. Therefore, assuming that the patient takes a bath 24 hours after application of a product without removing it, we performed the peeling off test after immersing the product in tepid water at 40°C for 30 minutes. As

a result, while Yutoku showed a significant increase of 1.47 N (p = 0.00074), Teikoku and Nippon-zoki showed significant decreases of 2.68 N (p = 2.7E-0.5) and 1.94 N (p = 2.3E-0.7), respectively. Since the peeling force increased in some products but decreased in others after immersion in tepid water at 40°C for 30 minutes, attention to the temperature at application is also considered necessary depending on the characteristics of the adhesive base used in each product. However, information concerning the constituent contents of each product obtained from the interview form was insufficient for thorough discussion. A large peeling force is a factor that contributes to detachment of the cuticle, other factors including "wetness" have also been reported to exert large effects (*13*). Therefore, in compliance guidance for patients, informing them that the tape can be removed more readily after a bath would be useful if a brand-name drug is changed to a generic drug such as Teikoku and Nippon-zoki.

From these results, in changing a brand-name product to a generic product, the feeling of use may be affected by selecting a product shown to have significantly different physicochemical properties compared with the one that has been used. Since, as indicated by the results of this study, pharmaceutical and physicochemical properties differ widely among the brand-name and generic products, the feeling of their use is expected to vary, and it becomes possible to select products according to the feeling of use preferred by each patient. Thus, in changing brand-name tape products to generic products, anticipating changes in the feeling of use and providing information based on the characteristics of each product are considered to contribute to the establishment of a relationship of trust between patients and medical staff.

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Original Article

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A novel highly concentrated enteral nutrition formula, EN-P05, shows nutritional effectiveness comparable to the approved OSN-001 in gastrostomized rats

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Summary **Enteral nutrition is beneficial support administered as oral supplements or *via* tube feeding for patients with long-term inability to meet nutritional requirements orally. However, because of the high volumes administered, vomiting and gastroesophageal reflux are often encountered in patients receiving enteral nutrition. EN-P05 is a novel, highly concentrated enteral nutrition formula that was developed to reduce dosing volume and that satisfies the Japanese recommended daily allowance for most vitamins and trace elements, even in patients who require low-calorie control, such as home-care patients. However, whether EN-P05 can provide nutritional management equivalent to that provided by approved formulas has remained unknown. To investigate the nutritional effectiveness of EN-P05, we evaluated body weight gain, serum chemistry parameters, nitrogen balance, and fat absorption in 7-week-old gastrostomized rats that received either EN-P05 or OSN-001 for 2 weeks. No difference in organ or carcass weight was found between the groups. No significant between-group differences were observed in serum albumin, total protein, triglycerides, or total cholesterol, nor in nitrogen retention or fat absorption rate. No adverse effects associated with administration of EN-P05 were found. These results suggest that EN-P05 can provide the same nutritional management as approved formulas, even when administered in smaller volume.**

Keywords: Nutritional management, nitrogen balance, dietary fiber, trace element, carnitine

1. Introduction

Enteral nutrients are widely used for nutritional supplementation in patients after surgery and in patients who have long-term difficulty with oral ingestion, due to factors such as sequelae of stroke, neurological intractable diseases, and severe motor and intellectual disabilities (*1-5*). Approved enteral nutrition formulas are designed to satisfy the daily vitamin and mineral requirements of adults consuming approximately 1,600 kcal/day in Japan. Therefore, patients who require long-term nutrient administration and who have low activity, with maintenance energy requirements of approximately 1,000 kcal/day, need to adjust their intake volume to meet the recommended daily caloric intake. However, such adjustment may result in deficiency of some vitamins or trace elements (*6-8*). It has also been reported that deficiency may result from administration of enteral nutrition formulas that do not contain trace elements (iodine, selenium, chromium, and molybdenum) newly categorized as essential in "Dietary Reference Intakes for Japanese (2000)" (*9-13*).

In addition to these nutritional management issues, the following goals must be met in clinical practice: reduction of volume to lower the risk of aspiration, securing time for rehabilitation by shortening administration time, and use of oral nutritional supplements for nutritional treatment (*14-18*). Therefore, a high-concentration enteral nutrition formula that allows efficient ingestion of energy and nutrients in smaller volumes is required.

Created to address these problems and the needs of medical personnel, patients, and caretakers, EN-P05 is a novel, high-concentration enteral nutrition formula with

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a caloric density of 1.6 kcal/mL developed to satisfy the recommended daily allowance of most vitamins and trace elements in accordance with "Dietary Reference Intakes for Japanese (2015)" at a dosage of 900 kcal/day. Moreover, EN-P05 also includes carnitine, which is reported to be deficient in patients with severe physical and mental disabilities and neuromuscular diseases who require long-term tube feeding (*19,20*).

In this study, EN-P05 and OSN-001 (an approved enteral nutrition formula) were administered to gastrostomized rats for 2 weeks, and their effects on nutritional status were compared to evaluate the nutritional effectiveness of EN-P05.

2. Materials and Methods

2.1. *Enteral nutrition formulas*

EN-P05 and OSN-001 (RACOL®-NF) were prepared at EN Otsuka Pharmaceutical Co., Ltd. (Hanamaki, Japan). The composition of each enteral nutrition formula is shown in Table 1.

Table 1. Nutritional composition of EN-P05 and OSN-001

Items	EN-P05 (/100 mL)	OSN-001 (/100 mL)
Protein (g)	6.40	4.38
Fat (g)	5.15	2.23
Carbohydrate (g)	21.22	15.62
Fiber (inulin[a]) (g)	1.6	-
Minerals		
Sodium (mg)	144.0	73.8
Potassium (mg)	294	138
Calcium (mg)	142.2	44.0
Magnesium (mg)	65.8	19.3
Phosphorus (mg)	177.8	44.0
Chloride (mg)	222	117
Iron (μg)	1,955	625
Zinc (μg)	2,133	640
Manganese (μg)	710	133
Copper (μg)	160	125
Iodine (μg)	23.0	-
Selenium (μg)	9.0	-
Chromium (μg)	7.0	-
Molybdenum (μg)	5.3	-
Vitamins		
Vitamin A (μg RE)	151.0	62.1
Vitamin D (μg)	2.67	0.34
Vitamin E (mg)	2.67	0.44
Vitamin K (μg)	13.33	6.25
Vitamin B1 (μg)	249	380
Vitamin B2 (μg)	285	245
Vitamin B6 (μg)	250	375
Vitamin B12 (μg)	0.80	0.32
Vitamin C (mg)	35.6	28.1
Nicotinamide (mg)	2.67	2.50
Pantothenic acid (μg)	1,067	958
Folic acid (μg)	42.7	37.5
Biotin (μg)	8.90	3.86
Carnitine (mg)	26.7	-
Choline (mg)	97.8	-
Energy (kcal)	160	100

[a]: artificially synthesized inulin-type fructans with a polymerization degree of approximately 6 to 30 fructose molecules. RE, retinol equivalents.

2.2. *Animals*

Six-week-old male Sprague-Dawley rats (Charles River Laboratories Japan, Inc., Yokohama, Japan) were housed in stainless metabolism cages (CT10-S; CLEA Japan, Inc., Tokyo, Japan) under controlled temperature (23 ± 3°C) and humidity (50 ± 20%), with a 12-hour light-dark cycle. The rats were fed commercial laboratory chow (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and were allowed drinking water *ad libitum* for about 1 week before the experiments began. All animal experiments conformed to the guidelines for the care and use of laboratory animals established by the Animal Use and Care Committee of EN Otsuka Pharmaceutical Co., Ltd.

2.3. *Surgical procedure for gastrostomy*

The gastrostomy surgical procedure followed the method of Murakami *et al*. (*21*). After an overnight fast, the rats were anesthetized with isoflurane (Pfizer Inc., New York, NY, USA). Laparotomy was performed through a midline incision. An 8-Fr catheter (Terumo Corporation, Tokyo, Japan) was then inserted into the stomach. The distal end of the catheter was tunneled subcutaneously through the left lateral abdominal wall, and exited at the interscapular region. The proximal end of the catheter was attached to a swivel spring, which allowed the rats freedom of movement in their individual cages. Penicillin G potassium (2,000 U; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) was intraperitoneally administered after laparotomy.

2.4. *Experimental design*

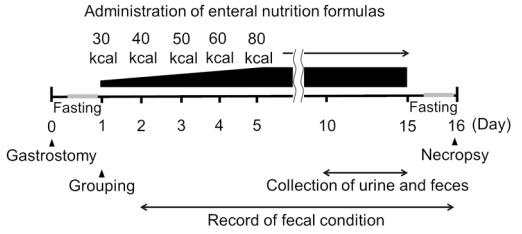

Figure 1. Experimental design. After gastrostomy, the rats received enteral nutrition in the amounts of 30, 40, 50, 60, and 80 kcal on Days 1, 2, 3, 4, and 5 to 14, respectively. After completion of administration of enteral nutrient, the animals were fasted overnight and necropsied the next day.

The experimental design is schematically outlined in Figure 1. The day after gastrostomy surgery, the rats were randomized into two groups, pair-matched according to body weight on Day 1: the EN-P05 group (n = 10) and the OSN-001 group (n = 10). The rats were administered EN-P05 or OSN-001 for 14 days *via* an infusion pump (SP-115; JMS Co., Ltd., Tokyo, Japan)

over approximately 16.5 h per day, beginning at 17:00. Taking the effects of their postoperative condition into account, the administered volume of enteral nutrition formulas was gradually increased over time. The fecal condition of each rat was recorded on Days 2 to 16, according to the following descriptions: no feces, normal feces, loose feces, watery feces. Urine and feces were collected on Days 10 to 15 (total of 5 days); the feces were lyophilized and weighed.

2.5. *Blood collection and measurement of organ weight*

On Day 16, blood samples were obtained with a vacuum blood collection tube (Terumo Corporation) from the vena cava of all rats under isoflurane anesthesia. After euthanasia, all rats were necropsied and organs were removed and weighed.

2.6. *Clinical chemistry analysis*

All blood samples for clinical chemistry analyses were centrifuged at 1,500× g for 15 minutes at 4°C, after which the serum was collected. Serum albumin, total protein, triglycerides, and total cholesterol were quantified with a clinical chemistry analyzer (Fuji DRI-CHEM 3500V; Fujifilm Medical Co., Ltd., Tokyo, Japan), in accordance with the manufacturer's instructions.

2.7. *Nitrogen balance*

The amount of nitrogen in urine and feces collected over a 5-day period and in the two enteral nutrition formulas was measured with the general Kjeldahl method. Using the obtained values, nitrogen retention, biological value, nitrogen retention rate, and apparent nitrogen absorption rate were calculated using the following formulas:

Nitrogen retention (mg/5 days) = Nitrogen intake – Urinary nitrogen excretion – Fecal nitrogen excretion

Biological value (%) = 100 × Nitrogen retention/(Nitrogen intake – Fecal nitrogen excretion)

Nitrogen retention rate (%) = 100 × Nitrogen retention/Nitrogen intake

Apparent nitrogen absorption rate (%) = 100 × (Nitrogen intake – Fecal nitrogen excretion)/Nitrogen Intake

2.8. *Fat absorption*

The amount of fat in feces collected over a 5-day period and in the two enteral nutrition formulas was measured using an acid decomposition method. Briefly, the samples were digested with hydrochloric acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and fat was extracted in an organic solvent containing diethyl ether, petroleum ether, and ethanol (Wako Pure Chemical Industries, Ltd.) at a ratio of 5:5:2. After centrifugation at 76× g, the supernatant was obtained and evaporated by heating; the residue was weighed as fat. Using the obtained values, the amount of apparent fat absorption and apparent fat absorption rate were calculated using the following formulas:

Apparent fat absorption (mg/5 days) = Fat intake – Fecal fat excretion

Apparent fat absorption rate (%) = 100 × (Fat intake – Fecal fat excretion)/Fat intake

2.9. *Statistical analysis*

The results are expressed as means ± SD. Statistical analysis was performed using one-way ANOVA. The frequency of recorded fecal conditions in each group was calculated and a chi-square test was conducted. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. *Changes in body weight and organ weight in gastrostomized rats administered each enteral nutrition formula*

First, to investigate the effect of EN-P05 on the growth of rats, body weight was measured during the period of enteral-nutrient administration and the weight gain from Day 1 to Day 15 was calculated. Body weight change of the EN-P05 group remained slightly higher compared to the OSN-001 group (Figure 2). Body weight and weight gain were significantly higher in the EN-P05 group than in the OSN-001 group on Day 15, the end date of enteral-nutrient administration. However, there was no significant difference between groups in body weight on Day 16, the day of necropsy after an overnight fast. Moreover, there were no differences between groups in the weight of the carcass, liver, kidney, spleen, or fat tissues on the day of necropsy (Table 2). Notably, the cecum with its contents was approximately twice as heavy in the EN-P05 group as in the OSN-001 group. These results indicated that the body weight gain in the

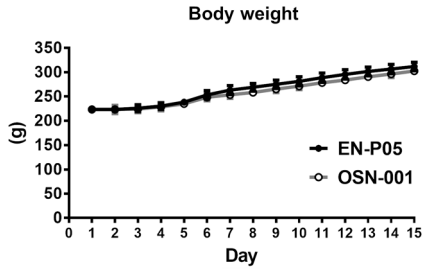

Figure 2. Body weight change of gastrostomized rats throughout the experiment (Days 1 to 15). Individual rats were weighed daily. The results shown are expressed as means ± SD (*n* = 10/group). Body weight was recorded as the weight of the rat with a gastrostomy catheter left *in situ*.

EN-P05 group was caused by an increase in the weight of the cecum and its contents.

3.2. *Nutritional markers in serum*

Next, a clinical chemistry analyzer was used to check nutritional markers in serum to investigate the effect of enteral nutrition formulas on nutritional status. There was no difference in serum albumin, and no significant difference in total protein concentration, serum triglycerides, or total cholesterol between groups (Table 3).

Table 2. Nutritional parameters in gastrostomized rats

Items	EN-P05 group	OSN-001 group
Body weight (g)		
Day 1	223.30 ± 6.38	223.31 ± 6.36
Day 15	311.93 ± 8.90**	302.70 ± 7.04
Day 16	289.25 ± 8.67	282.49 ± 5.63
Weight gain, Days 1-15	88.63 ± 6.67**	79.39 ± 5.85
Organ weight (g)		
Liver	8.25 ± 0.28	8.42 ± 0.38
Kidney	2.13 ± 0.12	2.07 ± 0.12
Spleen	0.64 ± 0.08	0.62 ± 0.10
Epididymal adipose tissue	2.97 ± 0.36	3.13 ± 0.44
Perinephric adipose tissue	3.67 ± 0.36	3.59 ± 0.64
Cecum	6.57 ± 1.40**	3.57 ± 1.53
Carcass (g)	215.99 ± 8.05	214.03 ± 8.51

Results shown are mean ± SD (n = 10/group). Carcass weight was determined as weight of rat body with all organs removed except the head; the cecum was weighed with its contents. Statistical significance was determined using one-way ANOVA (**$p < 0.01$).

Table 3. Serum biochemistry in gastrostomized rats

Items	EN-P05 group	OSN-001 group
Albumin (g/dL)	3.8 ± 0.3	3.8 ± 0.4
Total protein (g/dL)	5.3 ± 0.3	5.2 ± 0.3
Triglycerides (mg/dL)	83 ± 20	98 ± 24
Total cholesterol (mg/dL)	59 ± 9	59 ± 8

Results shown are mean ± SD (n = 10/group). Statistical significance was determined using one-way ANOVA.

Table 4. Nitrogen balance in gastrostomized rats

Items	EN-P05 group	OSN-001 group
Nitrogen intake (mg/5 days)	2,550.48	2,782.76
Fecal nitrogen excretion (mg/5 days)	227.36 ± 49.24**	179.46 ± 32.78
Urinary nitrogen excretion (mg/5 days)	1,336.50 ± 116.07**	1,708.00 ± 120.07
Nitrogen retention (mg/5 days)	986.62 ± 100.50	895.30 ± 130.54
Biological value (%)	42.49 ± 4.43**	34.37 ± 4.82
Nitrogen retention rate (%)	38.68 ± 3.94**	32.17 ± 4.69
Apparent nitrogen absorption rate (%)	91.09 ± 1.93**	93.55 ± 1.18

Results except nitrogen intake are shown as the mean ± SD (n = 10/group). Statistical significance was determined using one-way ANOVA (**$p < 0.01$).

3.3. *Nitrogen balance*

To explore the details of protein nutritional status, the nitrogen balance was assessed over a 5-day period, from Day 10 to Day 15. Nitrogen intake over the 5 days was about 200 mg higher in the OSN-001 group than in the EN-P05 group, and fecal nitrogen excretion was significantly higher in the EN-P05 group than in the OSN-001 group (Table 4). Thus, the apparent nitrogen absorption rate was lower in the EN-P05 group than in the OSN-001 group. However, because urinary nitrogen excretion was significantly higher in the OSN-001 group than in the EN-P05 group, the biological value and nitrogen retention rate were significantly lower in the OSN-001 group than in the EN-P05 group.

3.4. *Fat absorption*

Next, fat absorption was examined to investigate the state of lipid nutrition in detail. Fat intake over a 5-day period was about 4 g higher in the EN-P05 group than in the OSN-001 group, and fecal dry weight was significantly higher in the EN-P05 group than in the OSN-001 group (Table 5). Apparent fat absorption over a 5-day period was higher in the EN-P05 group than in the OSN-001 group, whereas the apparent fat absorption rate was very similar between the groups. These data suggest that the difference in apparent fat absorption directly reflected the difference in fat intake.

3.5. *Fecal condition in gastrostomized rats administered each enteral nutrition formula*

As EN-P05 is a highly concentrated enteral nutrition formula, whether it would adversely affect the fecal condition of rats with gastrostomy was investigated. No rats in either group had watery feces during the study period; however, the frequencies of loose feces and fecal condition patterns were significantly different between groups (Table 6). Focusing on temporal changes in fecal condition, no difference between groups was found in the fecal condition pattern during the period from Days 2 to 5, in which the administered

Table 5. Fat absorption in gastrostomized rats

Items	EN-P05 group	OSN-001 group
Fat intake (g/5 days)	12.73	8.80
Fecal fat excretion (g/5 days)	0.41 ± 0.12*	0.29 ± 0.06
Fecal dry weight (g/5 days)	3.34 ± 0.80**	2.00 ± 0.38
Apparent fat absorption (g/5 days)	12.32 ± 0.12**	8.51 ± 0.06
Apparent fat absorption rate (%)	96.79 ± 0.93	96.68 ± 0.66

Results except fat intake are shown as the mean ± SD (n = 10/group). Statistical significance was determined using one-way ANOVA (*$p < 0.05$, **$p < 0.01$).

Table 6. Fecal conditions in each period in gastrostomized rats

Items	Frequency		*p* value
	EN-P05 group	OSN-001 group	
Days 2 to 16:			< 0.01
No feces	5 (3.3)	10 (6.7)	
Normal feces	32 (21.3)	5 (3.3)	
Loose feces	113 (75.3)	135 (90.0)	
Watery feces	0 (0.0)	0 (0.0)	
Days 2 to 5:			0.57
No feces	4 (10.0)	6 (15.0)	
Normal feces	5 (12.5)	5 (12.5)	
Loose feces	31 (77.5)	29 (72.5)	
Watery feces	0 (0.0)	0 (0.0)	
Days 6 to 16:			< 0.01
No feces	1 (0.9)	4 (3.6)	
Normal feces	27 (24.5)	0 (0.0)	
Loose feces	82 (74.5)	106 (96.4)	
Watery feces	0 (0.0)	0 (0.0)	

Data are shown as number of observations (percentage). Statistical significance was determined with the chi-square test for only three items (No feces, Normal feces, and Loose feces) between the groups, because the frequency of Watery feces in each period was 0.

dose of enteral nutrition formula was gradually increased. After the maximum dose of enteral nutrients was achieved on Day 6, normal feces were observed in the EN-P05 group at a frequency of approximately 25%, whereas no normal feces were observed in the OSN-001 group.

4. Discussion

In the present study, the effectiveness of a novel highly concentrated enteral nutrient, EN-P05, was compared to that of an approved one, OSN-001, in gastrostomized rats. There was a significant between-group difference in weight gain, which could be due to an increase in cecum weight in the EN-P05 group. Several studies have reported that dietary fiber causes hypertrophic changes in the cecum and increases fecal volume in rats, without causing toxicity (*22*). The water-soluble dietary fiber, inulin, which is present in EN-P05, may therefore have caused the increased cecum weight in the EN-P05 group. The finding of no difference between groups in the weights of other organs suggests that there was no substantial body weight difference between groups.

In regard to nitrogen balance, the biological value and nitrogen retention rate were significantly higher in the EN-P05 group than the OSN-001 group. It has been reported that differences in protein content among enteral nutrition formulas are associated with differences in urinary nitrogen excretion in rats, and that the biological value and nitrogen retention rates change in relation to these differences (*23-25*). Therefore, the differences in the biological value and nitrogen retention rates in the present study may be attributable to the use of two enteral nutrition formulas with different protein content. Moreover, the apparent nitrogen absorption rate was significantly lower in the EN-P05 group than the OSN-001 group. This result may be explained by higher nitrogen excretion into feces because of the inulin contained in EN-P05, which is a dietary fiber known to increase the excretion of exogenous and endogenous nitrogen (*26-29*). Although the three parameters described above differed between the groups, there was no such difference in the total amount of nitrogen retention, serum albumin concentration, or total protein concentration. Therefore, we consider that protein nutritional status was equivalent in the two groups.

Regarding fat absorption, there was a between-group difference in apparent fat absorption, but not in the actual weight of epididymal or perinephric adipose tissue at the time of necropsy, and not in triglycerides or total cholesterol concentration in serum. These data suggest that lipid nutritional status was equivalent in the two groups.

In this study, EN-P05-administered gastrostomized rats had better fecal condition than OSN-001-administered gastrostomized rats. Of note, normal feces were observed in the EN-P05 group at a frequency of approximately 25%, but were not observed in the OSN-001 group from Day 6. The fecal condition might be influenced by the amount of moisture present in each formula. Because OSN-001 is less concentrated than EN-P05, and the amount of water per unit of energy is larger, it is conceivable that the larger volume of fluid flowing into the intestinal tract in the OSN-001 group caused the increased frequency of loose feces. It is also possible that the observed differences in fecal status may involve dietary fiber. Fermentation of dietary fibers by gut microbiota yields short-chain fatty acids, which provide an energy source for colonic epithelial cells, enhance water absorption from colonic epithelium, and control mucosal immunity (*30*). Moreover, high concentration of short-chain fatty acids helps to maintain low pH in the colon for colonic microbiota and prevents infection by enteropathogenic organisms that can cause diarrhea (*31*). These findings suggest that inulin, a dietary fiber included in EN-P05, also promoted good fecal condition in the gastrostomized rats, *via* promotion of colonic water absorption and prevention of pathogen overgrowth resulting from production of short-chain fatty acids.

In summary, the nutritional effectiveness of EN-P05 versus OSN-001 when administered for 2 weeks to gastrostomized rats were compared. There were no differences between the EN-P05 group and OSN-001 group in body weight, organ weight, blood biochemical test values, nitrogen retention, or fat absorption rate. Additionally, there were no adverse effects associated with administration of EN-P05. These results suggest that EN-P05 is as nutritionally effective as OSN-001, and as useful for nutritional management as

conventional enteral nutrition formulas, even in a lower administration volume.

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Case Report

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Rapid temporal improvement of pembrolizumab-induced pneumonitis using the anti-TNF-α antibody infliximab

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Summary **Immune checkpoint inhibitors are associated with a wide spectrum of immune-related adverse events (irAEs) that are typically transient but are sometimes severe or even fatal. No consensus exists for the treatment of severe immune-mediated pneumonitis that is refractory to corticosteroids. Here, we report an autopsy case of pembrolizumab-induced pneumonitis that was transiently improved using infliximab. A 67-year-old male with advanced lung adenocarcinoma developed pneumonitis two weeks after a single dose of first-line pembrolizumab. The pneumonitis was refractory to corticosteroids, and the patient required mechanical ventilation. Addition of a single dose of infliximab rapidly improved the respiratory status and chest CT showed resolution of ground-glass opacities in the right upper and middle lobes. However, the patient died from re-exacerbation of pneumonitis 17 days after infliximab administration. The autopsy confirmed organizing phase diffuse alveolar damage in the right lower lobe, while the right upper lobe remained almost intact consistent with the CT findings, which is suggestive of the therapeutic effect of infliximab. The half-life of infliximab is 7-12 days, and a second dose of infliximab two weeks after the first dose is sometimes required for the treatment of gastrointestinal toxicity induced by anti-CTLA4 antibodies. Although the current guidelines do not recommend repeated administration of infliximab for immune-mediated pneumonitis, the present case suggests that repeated infliximab therapy may be beneficial in the treatment of immune-mediated pneumonitis.**

Keywords: Immune-related adverse events, diffuse alveolar damage, lung adenocarcinoma

1. Introduction

Pembrolizumab monotherapy has shown survival benefit as a first-line therapy for patients with metastatic non-small cell cancer (NSCLC) with a programmed death ligand 1 (PD-L1) tumor proportion score of 50% or greater and without *epidermal growth factor receptor* (*EGFR*) mutation or *anaplastic lymphoma receptor tyrosine kinase* (*ALK*) translocation (*1*). The addition of pembrolizumab to pemetrexed and a platinum-based chemotherapy also prolongs overall survival in patients with metastatic non-squamous NSCLC without *EGFR* or *ALK* alterations regardless of the tumor proportion score (*2*).

Although pembrolizumab is generally well-tolerated compared to cytotoxic chemotherapy, immune-related adverse events (irAEs) are sometimes severe or even fatal. The severity of irAEs is graded according to the Common Terminology Criteria for Adverse Events (CTCAE) (*3*). The incidence of immune-mediated pneumonitis of any grade was 5.8% and that of grade ≥ 3 was 2.6% in the phase 3 KEYNOTE-024 trial (*1*). For grade ≥ 3 pneumonitis, the guidelines of the European Society for Medical Oncology (ESMO) (*4*) and American Society of Clinical Oncology (*5*) recommend intravenous methylprednisolone at 2-4 mg/kg/day and

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1-2 mg/kg/day, respectively. If pneumonitis does not improve within 48 hours, the guidelines recommend addition of an immunosuppressant such as infliximab, mycophenolate mofetil or cyclophosphamide (*4,5*). The guidelines also recommend gradual tapering of corticosteroids over at least six weeks. In contrast, there are no guidelines for the repeated administration of immunosuppressants for immune-mediated pneumonitis. The increasing use of immune-checkpoint inhibitors requires the accumulation of case reports to determine the best management strategies for severe immune-mediated pneumonitis.

2. Case Report

A 67-year-old man with a 45-pack-year history of tobacco use presented with a massive left pleural effusion. He had undergone sigmoidectomy for colon cancer at the age of 37, and had had transient thyrotoxicosis at the age of 55. A chest computed tomography (CT) scan revealed a mass in the left upper lobe with left pleural effusion, enlarged mediastinal and contralateral hilar lymph nodes and intrapulmonary metastatic nodules in the right lung (Figure 1).

Adenocarcinoma cells were detected in the left pleural effusion and in the subcarinal lymph node tissue obtained using endobronchial ultrasonography-guided transbronchial needle aspiration. The adenocarcinoma cells in the subcarinal lymph node were positive for thyroid transcription factor 1 (TTF-1), and the patient was diagnosed with stage IVA (cT4M3M1a) lung adenocarcinoma (*6*).

The tumor was negative for both *EGFR* mutation and *ALK* and *ROS1* rearrangement. The tumor proportion score in the 22C3 PD-L1 assay was 95% in the subcarinal lymph node. The patient had no obvious past history of autoimmune disease, and the chest CT scan showed no underlying interstitial pneumonia, with normal serum KL-6 levels. Although anti-thyroid antibodies (anti-thyroglobulin and anti-thyroid peroxidase) were both positive and a thyroid ultrasound revealed chronic thyroiditis, thyroid function test parameters, including thyroid-stimulating hormone, free T4 and T3, were all normal.

After drainage of the left pleural cavity, the patient received 200 mg pembrolizumab as first-line treatment. On day 5, the patient presented with an erythematous maculopapular rash on the body trunk. The skin irAE was grade 2 (*3*), and resolved one week later following topical corticosteroid and oral antihistamine treatment. On day 14, the patient developed acute respiratory failure requiring 10 L/min of oxygen, and chest CT showed bilateral non-segmental ground-glass opacities (Figures 2A and 2B). The patient was diagnosed with immune-mediated pneumonitis, and administration of a high-dose intravenous corticosteroid (125 mg methylprednisolone) with a broad spectrum antibiotic for possible bacterial infection was initiated.

On day 15, however, the patient was transferred to the intensive care unit with worsening hypoxia requiring mechanical ventilation. As pneumonitis was refractory to corticosteroid treatment (250 mg methylprednisolone on days 15-17), we administered 5 mg/kg infliximab on day 17. The addition of infliximab

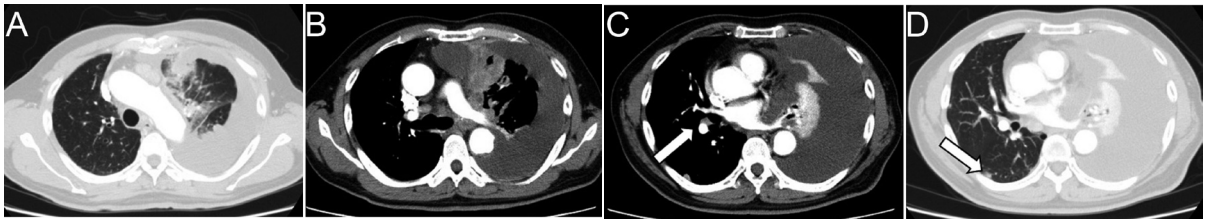

Figure 1. Chest CT scan on admission. Primary tumor in the left upper lobe showed direct invasion into the mediastinum with left pleural effusion and pleural nodules **(A** and **B)**. Contralateral hilar lymph node metastasis (**C**, arrow) and contralateral pulmonary metastasis (**D**, arrow) were also suspected.

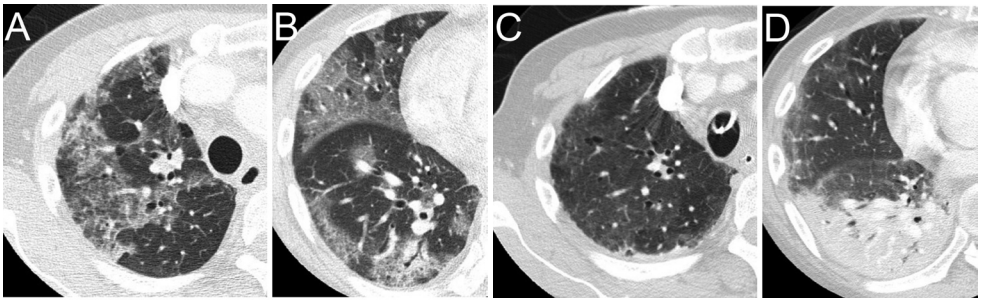

Figure 2. Radiological response of immune-mediated pneumonitis to infliximab. Chest CT scan on day 14 showed non-segmental diffuse ground-glass opacities consistent with immune-mediated pneumonitis **(A** and **B)**. Chest CT scan on day 23 demonstrated resolution of the ground-glass opacities in the right upper lobe **(C)**, and consolidation in the dependent part of the right lower lobe **(D)**.

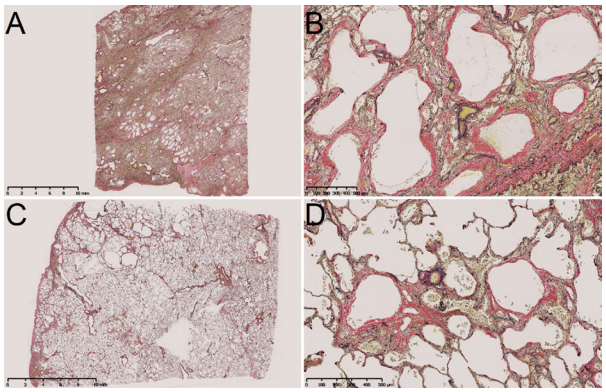

Figure 3. Autopsy findings. The right lower lobe showed organizing phase diffuse alveolar damage with extensive collapse of alveolar spaces **(A** and **B)**. In contrast, the lung parenchyma remained mostly intact with focal organization in the right upper lobe **(C** and **D)**.

rapidly improved respiratory status, and the PaO2/FiO2 ratio increased from 91 mmHg (day 16) to 169 mmHg (day 21). Although the consolidation in the right lower lobe did not improve, the resolution of the ground-glass opacities in the right upper and middle lobes was observed on day 23. (Figures 2C and 2D). The intravenous methylprednisolone dose was gradually tapered from 125 mg on days 18-20, to 60 mg on days 21-27, and 50 mg on day 28 and thereafter. On day 31, however, the patient's respiratory status deteriorated again and he died on day 34.

Autopsy revealed organizing phase diffuse alveolar damage in the right lower lobe with extensive collapse of alveolar spaces (Figures 3A and 3B). In contrast, the right upper lobe remained mostly intact with focal organization (Figures 3C and 3D). No infectious etiology was identified, and the tumor in the left lung showed no pathological response to pembrolizumab.

3. Discussion

The radiological pattern of immune-mediated pneumonitis are associated with mortality and response to corticosteroid. In the retrospective report by Nishino *et al*. (*7*), 65% of patients (13/20) had a cryptogenic organizing pneumonia (COP) pattern, and none of them died (0/13). All but one patient with COP pattern were treated with only corticosteroids, while the one patient was administered a corticosteroid and infliximab. Acute interstitial pneumonia (AIP)/acute respiratory distress syndrome (ARDS) pattern was observed in 10% (2/20) of patients. Although the two patients with the AIP/ARDS pattern were treated with corticosteroid and infliximab (5 mg/kg), one patient died.

In the current case, the pembrolizumab-induced pneumonitis with AIP/ARDS pattern on CT (*8*) did not respond to corticosteroid treatment, but respiratory status improved one week after administration of infliximab. Although consolidation in the right lower lobe showed no response to infliximab, the improvement in the right upper lobe observed by CT were confirmed by autopsy.

There is no consensus on the dosing schedule of infliximab for the treatment of severe immune-mediated pneumonitis. Based on a retrospective report (*9*), the current ESMO guideline states that gastrointestinal toxicity induced by anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibodies may require a second dose of infliximab two weeks after the first administration (*4*). To our knowledge, the present case is the first report of immune-mediated pneumonitis that demonstrated transient improvement and re-exacerbation two weeks after infliximab therapy. Considering that infliximab has a half-life of 7-12 days (*10*), elimination of infliximab might have led to re-exacerbation of the pneumonitis in the current patient.

In conclusion, infliximab induced rapid improvement of immune-mediated pneumonitis that lasted for two weeks. Repeated administration of infliximab for a certain period may be beneficial in the treatment of immune-mediated pneumonitis. The present case highlights the need for further research on the choice and optimal dose and duration of immunosuppressants in the treatment of severe immune-mediated pneumonitis.

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Case Report

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Efficacy of trazodone for treating paroxysmal sympathetic hyperactivity presenting after thalamic hemorrhage: A case report

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Summary **Paroxysmal sympathetic hyperactivity (PSH) is a clinical condition characterized by abnormal paroxysmal surges in sympathetic nervous system activity. PSH is known to occur after severe head injury and hypoxic encephalopathy. Cases of PSH that develop after stroke have been reported worldwide; however, PSH is not commonly reported in the field of stroke research in Japan. Some studies have suggested that gabapentin may improve the symptoms of PSH. To our knowledge, this is the first case report demonstrating the efficacy of trazodone for the treatment of PSH that developed after thalamic hemorrhage. A 45-year-old woman presented to our clinic with headache and paralysis of the left side of her body after experiencing right thalamic hemorrhage; a conservative treatment was initiated at our hospital. Immediately upon hospitalization, she developed high fever, tachycardia, tachypnea, constipation, and overactive bladder and had breathing difficulties. Blood sampling revealed elevated levels of myocardial escape enzymes; however, coronary angiography did not show any significant stenosis or occlusion. The patient's symptoms improved after the administration of trazodone. She was diagnosed with catecholamine cardiomyopathy associated with PSH after intracranial hemorrhage and was subsequently transferred to a recovery and rehabilitation hospital unit where the oral administration of trazodone continued. Prolonged PSH contributes significantly to the impairment of daily activities in patients with stroke; therefore, early diagnosis and treatment are critical. Here, we report on the efficacy of trazodone as an effective treatment option for improving clinical outcomes and reducing the stay in the stroke care unit.**

Keywords: Paroxysmal sympathetic hyperactivity, thalamic hemorrhage, trazodone

1. Introduction

Paroxysmal sympathetic hyperactivity (PSH) is a clinical condition characterized by abnormal paroxysmal surges in sympathetic nervous system activity. Although the symptoms of PSH have been identified for longer than 60 years, it has had over 31 different names, including dysautonomia, paroxysmal autonomic instability with dystonia, paroxysmal sympathetic storm, sympathetic storm, autonomic storm, diencephalic seizure, and autonomic dysfunction syndrome, to name a few, which makes it very difficult to identify (*1,2*). PSH often occurs after severe head injury and hypoxic encephalopathy, although it is also known to develop after stroke. However, in Japan, limited evidence regarding a connection between PSH and stroke exists (*3,4*). Therapeutic drugs, including morphine, benzodiazepines, beta-blockers, baclofen, gabapentin, and clonidine, are commonly used to suppress PSH. The inadequate therapeutic effect of these drugs necessitates the inclusion of bromocriptine (a dopamine agonist) to the treatment regimen (*5-8*). However, evidence suggests that such treatment regimens are therapeutically

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ineffective (*7*). Moreover, antiepileptic drugs are generally ineffective for treating PSH. Alternatively, multiple papers have reported on the efficacy of gabapentin for treating PSH (*1-3,5,9,10-13*), which is considered to improve the symptoms by controlling the suppressive nerve stimulation (*5*). However, there is no report on the therapeutic effect of sympathetic blockers, *i.e.*, α blockers. To our knowledge, the efficacy of trazodone for treating PSH that developed after thalamic hemorrhage has not been reported. Here, we describe the case of a patient who developed PSH after right thalamic hemorrhage, which was successfully treated with trazodone.

2. Case Report

A 45-year-old woman presented to our clinic with headache and left paralysis after a right thalamic hemorrhage. She had a medical history of hypertension but did not use any antihypertensive medication. Neurological assessments revealed paralysis and sensory disturbances in her left upper and lower limbs and left central facial paralysis. She scored 8/42 on the National Institutes of Health Stroke Scale; her modified Rankin scale score at admission was 3, and her blood pressure at hospitalization was 258/143 mmHg, indicative of a hypertensive emergency. Electrocardiography and blood analyses, including blood cell counts, biochemistry, and coagulation parameters, revealed no abnormalities. A plain head computed tomography showed right thalamic hemorrhage (Figure 1A), and susceptibility-weighted magnetic resonance imaging showed cerebral microbleeds in the contralateral basal ganglia without any blood vessel malformations (Figure 1B). Magnetic resonance angiography revealed no aneurysms or blood vessel malformations (Figure 1C), and a chest radiograph showed no apparent heart expansion or pulmonary edema (Figure 1D).

The patient was administered nicardipine as a conservative treatment for management of her blood pressure; the targeted systolic blood pressure was ≤ 140 mmHg. Immediately upon hospitalization, the patient developed a sudden high fever, accompanied by mass sweating, tachycardia, a significant increase in blood pressure, tachypnea, constipation, overactive bladder, and breathing difficulties. Head computed tomography images showed no enlargement of the hematoma on day 1 of hospitalization, and she was prescribed azilsartan (40 mg/day), amlodipine (10 mg/day), and trichlormethiazide (2 mg/day) to manage the hypertension.

On day 3 of hospitalization, the patient did not report chest pain but had difficulty breathing and insomnia. Blood sampling revealed elevated levels of myocardial escape enzymes, including troponin T, 0.34 ng/mL; creatine kinase, 446 U/L; lactate dehydrogenase, 292 U/L; adrenaline, 68 pg/mL; noradrenalin, 851 pg/mL; dopamine, 25 pg/mL; renin activity, 3.0 ng/mL/h; aldosterone, 183 pg/mL; and cortisol, 21.6 μg/dL. All cardiac parameters were above the normal levels, except adrenaline. No abnormalities were observed on electroencephalography; however, it showed ST-segment and T-wave changes in leads I, II, aVL, V5, and V6 (Figure 2A). Cardiac ultrasonography, performed on day 3 of hospitalization,

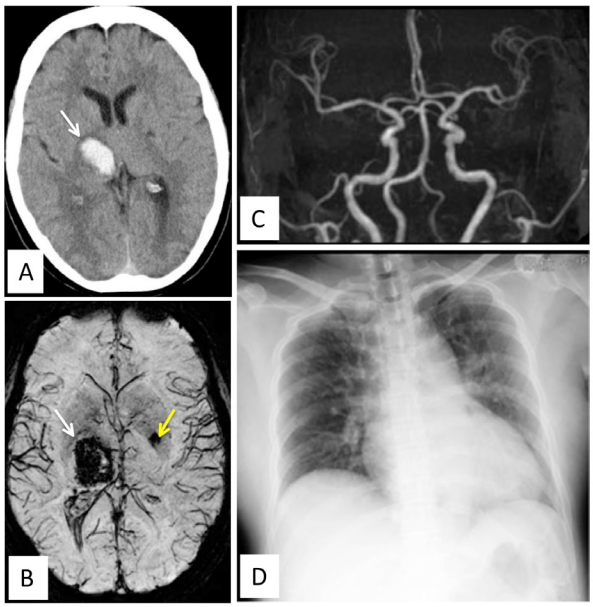

Figure 1. Clinical imaging for stroke signs upon initial presentation. (A) Head plane computed tomography image reveals a right thalamic hemorrhage (white arrowhead). **(B)** Susceptibility-weighted magnetic resonance image shows cerebral microbleeds (yellow arrowhead) in the left basal ganglia but no blood vessel malformations (white arrowhead). **(C)** Magnetic resonance angiography image reveals no aneurysms or blood vessel malformations. **(D)** Chest radiography shows no apparent heart expansion or pulmonary edema.

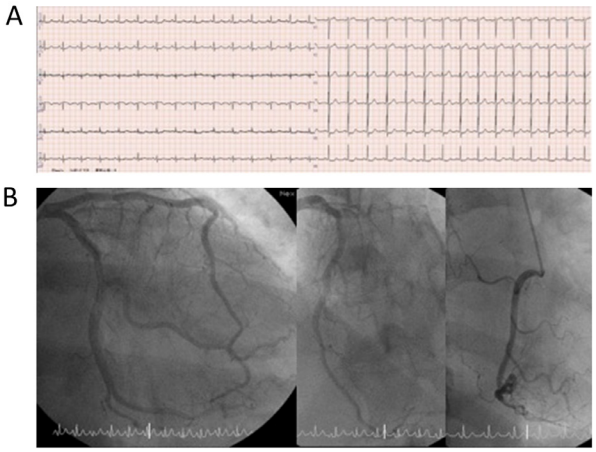

Figure 2. Examinations performed on days 5 and 9 of hospitalization. (A) Electrocardiography performed on day 5 shows ST-segment and T-wave (ST-T) changes in leads I, II, aVL, V5, and V6. **(B)** Coronary angiography images acquired on day 9 do not show any significant coronary artery stenosis or occlusion.

showed no wall motion abnormalities. Based on the series of systemic symptoms experienced, including the autonomic symptoms that were indicative of an intracranial hemorrhage associated with PSH, she was administered trazodone (50 mg/day), beginning on day 5 of hospitalization. As we had experienced a case in which trazodone was effective as a sympathetic blocker for Barré-Lièou syndrome (BLS), based on the post-traumatic sympathetic hyperactivity theory, we hoped that it would also be effective for PSH, which is a similar pathological condition.

Under this treatment regimen, the patient's breathing difficulties were rapidly alleviated and most of the other autonomic symptoms also improved. However, the periodic fever was sustained and was treated with an antipyretic, and she continued her rehabilitation without additional problems. Coronary angiography performed on day 9 of hospitalization showed no significant coronary artery stenosis or occlusion (Figure 2B), allowing the exclusion of acute coronary syndrome from the diagnosis. Because the patient's symptoms improved significantly after the administration of trazodone, she was diagnosed with catecholamine cardiomyopathy associated with PSH.

By day 10 of hospitalization, her general condition had stabilized, and she was moved from the stroke care unit to the general ward. She was transferred to a separate recovery rehabilitation hospital 20 days after admission, where the trazodone treatment (50 mg/day) continued. Trazodone was gradually reduced and eventually stopped 1 month after initial admission. No relapse of PSH was observed until 6 months after admission, and the dose of azilsartan was gradually decreased to 20 mg/day, and the patient had a modified Rankin scale score of 2 at the outpatient follow-up examination 10 months after admission. Written informed consent was obtained from the patient for publication of this case report and the accompanying images, and the study design was approved by the appropriate ethics review board.

3. Discussion

To our knowledge, the present report is the first to demonstrate the efficacy of trazodone for the treatment of PSH that developed after thalamic hemorrhage. PSH has only recently been defined (*1*), and is characterized by excessive autonomic symptoms, including high fever, high blood pressure, tachycardia, tachypnea, perspiration, and muscle tone abnormality. PSH occurs after severe brain injury, usually during a state of paroxysmal sympathetic excitement (*10*). Following paroxysmal excitement, the autonomic symptoms typically occur approximately five times a day, each episode lasting approximately 30 min. PSH causes hyperthermia, dehydration, muscle mass reduction, and muscle contracture and has a serious effect on reversion, such as symptom recurrence or prolonged requirement of intensive care unit management, or causes serious secondary sequelae (*5,11-13*). Although these complications can be avoided by early diagnosis and treatment (*6-8,14,15*), the detection of PSH is impossible without any knowledge of the underlying pathophysiology. Previously, the lack of a clear definition and diagnostic criteria resulted in poor understanding of the condition, and moreover, the variations in the symptoms complicated the diagnosis of PSH. In our case, we did not observe an epileptic wave on electroencephalography and antiepileptic drugs were not administered; therefore, a diagnosis of epilepsy was rejected. Since the series of her general symptoms resembled post-traumatic sympathetic hyperactivity to the prevailing BLS (*16*), we suspected PSH, and the diagnosis was confirmed once the symptoms met the known diagnostic criteria (*1*). We previously reported on the efficacy of trazodone for BLS (unpublished observations), and as PSH, similar to BLS, is a sympathetic condition, we assumed that trazodone use would be effective in this case.

Currently, there are two theories (*11,15*) that explain the pathophysiology of PSH. Specifically, it is theorized that the decoupling of the sympathetic excitement center of the hypothalamus and brainstem from the control of higher functioning brain regions, such as the cerebral cortex, results in a state of sympathetic excitement. The second theory suggests that when the midbrain or brainstem, regions that control afferent stimulation in the spinal cord, is injured, it becomes impossible to suppress the stimulation, which leads to hyperexcitability in the afferent pathway of the spinal cord. Currently, the latter theory has greater support (*11,15*).

Research has shown that PSH most commonly occurs in younger individuals; indeed, Hughes *et al*. (*15*) reported that the mean age of patients with PSH was 33.6 years, which is consistent with the age of our patient. Few reports of stroke-associated PSH in Japan have been published (*1,16*). Although the reason for this is unknown, awareness and understanding of the pathological condition are poor; therefore, it is possible that the occurrence of this condition has not been accurately reported.

The patient was diagnosed with PSH associated with intracranial hemorrhage. Interestingly, she often complained of respiratory distress. After assessing the coronary angiography, cardiac ultrasonography examination, blood sampling, and electrocardiography results, the patient was also diagnosed with catecholamine cardiomyopathy caused by PSH. To our knowledge, this is the first reported case of stroke-related PSH with catecholamine cardiomyopathy.

Trazodone, a well-known antidepressant drug widely used worldwide, works as a 5-hydroxytryptamine (5-HT2) and α1-adrenergic receptor antagonist and a

serotonin reuptake inhibitor (*17*). Symptoms improved after trazodone administration to patients with BLS and PSH, which are considered similar pathological conditions involving sympathetic hyperactivity, and we assume that trazodone's alpha blocking action was responsible for this effect. It was considered that this action could suppress sympathetic hyperactivity. There have been few reports of trazodone side effects, such as 270 cases of drowsiness (3.64%), 215 cases of dry mouth (2.90%), and 134 cases of constipation (1.81%) (*18*) and it is relatively safer for use in the elderly. In our case, trazodone, which is often used for treating depression because of its mechanism related to alpha-adrenoceptor inhibition (*17*), was effective for treating PSH.

Recognition of PSH is crucial for the rapid recovery of patients with traumatic brain injury or stroke, even when they are still in intensive care units. It is also important to reduce complication rates and the length of hospitalization (*6*). PSH is a common syndrome, and failure to recognize this condition is associated with increased morbidity and mortality, higher health costs, longer hospitalization, and poorer outcomes (*3*). In the present case, we believe that the early diagnosis and treatment of PSH, considering the symptoms of paroxysmal sympathetic hyperactivity that occurred after intracerebral hemorrhage, contributed to the overall short duration of hospitalization (in the stroke care unit and general hospital ward). In the future, it is desirable to accumulate more cases to conclusively comment on the efficacy of trazodone for PSH.

In conclusion, trazodone was effective in treating PSH in our case, and its use may reduce the overall duration of hospitalization and improve clinical outcomes in affected individuals. Trazodone can be an effective drug for PSH treatment, although further evidence accumulation from a larger number of cases is needed.

Acknowledgements

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Letter

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Immediate antiviral therapy for HIV-infected persons faces with various obstacles

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Summary **Human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) ranks eighth in the global burden of disease, making seriously threatens to global health. Given there is not yet a cure for HIV infection, antiretroviral therapy (ART) holds a key role not only in improving the prognosis of the patients, but also reducing the risk of HIV transmission. The immediate initiation of ART has been recommended in domestic and foreign policies and guidelines, yet the implementation of this strategy is not satisfactory. In developing countries and even in some developed countries, it still takes a long time for patients to go from the diagnosis of HIV infection to the acceptance of ART. Clarifying the obstacles to the implementation of immediate ART and finding strategies to cope with them have emerged as key problems in response to HIV/AIDS.**

Keywords: HIV/AIDS, antiviral therapy, health service quality

Human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) ranks eighth in the global burden of disease in 2017, making seriously threatens to global health (*1-5*). Due to its complexity and lethality, there are total 36.9 million people living with HIV and 1.8 million people newly infected with HIV in 2016 globally, and total 849,602 people with HIV/AIDS and 262,442 people AIDS-related deaths as of September 30, 2018 in China (*6,7*). Curbing the AIDS epidemic is an important part of the United Nations 2030 sustainable development goals (*8,9*), which still has considerable challenges.

Given there is not yet a cure for HIV infection, antiretroviral therapy (ART) holds a key role not only in improving the prognosis of the patients, but also reducing the risk of HIV transmission (*10-17*). The guidelines of World Health Organization (WHO), European AIDS Clinical Society (EACS), U.S. Department of Health and Human Services (DHHS) and Chinese Medical Association (CMA) all recommended that ART should be initiated in all HIV-infected adults, regardless of $CD4^+$ cell count (*18-20*).

However, the implementation of this strategy is not satisfactory. In developing countries and even in some developed countries, it still takes a long time for patients to go from the diagnosis of HIV infection to the acceptance of ART. The European Centre for Disease Prevention and Control (ECDC) report showed that only 16 of the 23 countries in Europe and Central Asia was reported to start treatment within one month of diagnosis (*21*); Oliver Bacon's study showed median time from diagnosis to first virologic suppression in San Francisco was 134 days in 2013 (*22*); Zunyou Wu's study showed the time from HIV confirmation to ART initiation was 53 days without intervention (23).

Clarifying the obstacles to the implementation of immediate ART has emerged as key problems in response to HIV/AIDS. The key obstacles in current research can be summarized in the following three aspects. *i*) Demand-side. Risk factors for delayed ART initiation include the key populations of men having sex with men (MSM), injecting drug users, male, older age, unmarried or divorced, *etc*., and the HIV-infection persons with long-term out-migrating/working,

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refusal of treatment, negative psychological emotions, treatment related costs, *etc*. (*24-26*). *ii*) Supply-side. Health system challenges are a barrier to getting people diagnosed with HIV onto treatment in many countries, including the insufficient knowledge and skills of health professionals, inadequate referral mechanism, weak confidentiality and availability of treatment options, *etc*. (*27,28*). *iii*) Intervention-side. This section includes the social and cultural issues, including the laws and policies, stigma and discrimination, tedious treatment process, *etc*. (*21,26,29*).

Nevertheless, due to the short emergence time of this phenomenon, few studies to provide systematic, quantitative and suitable countermeasures and suggestions for this issue. Finding the systematic and quantitative strategies to cope with these obstacles has important theoretical value and practical significance. Firstly, conduct more multi-center clinical trials to further promote the updating of guidelines. In 2017, WHO guidelines put forward the strategy of Rapid ART Initiation (*30*), suggesting that all HIV-infected persons must start ART rapidly (less than 7 days after HIV positive diagnosis); and for those ready to begin the treatment, ART should be initiated on the same day. But it also noted that medical resources are key constraints, so more clinical trials are needed. Secondly, except for identifying the key obstacles, revealing the underlying mechanisms of such obstacles and selecting the optimal coping strategies based on mechanism research also important for optimizing HIV prevention and control policies.

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Letter

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Brain abscess in an angiosarcoma patient during a disease-free interval

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Summary **This is the first case of an angiosarcoma patient with brain abscess, and it might be responsible for skin defect and cranial bone necrosis by surgical excision and radiation. Our patient was treated with 10 courses of triweekly paclitaxel therapy, radical radiotherapy (70 Gy), and surgical excision (2 cm margin apart from a lesion) for angiosarcoma. At two years after the operation he was diagnosed as brain abscess. Brain abscess was managed with antibiotic drugs and drainage, his clinical symptoms improved by these treatments. He achieves replace free survival without the exacerbation of angiosarcoma and brain abscess for three years.**

Keywords: Angiosarcoma, brain abscess, multimodality therapy

Although standard treatment strategies are yet to be established for scalp angiosarcoma, the current multimodality therapy generally consists of surgery, radiotherapy and chemotherapy (*1*). Skin defects due to radiotherapy and surgery often reduce quality of life. Here, we describe our first experience of an angiosarcoma patient with a brain abscess, and how it may be responsible for skin defects and cranial bone necrosis resulting from surgical excision and radiation.

A 76 year-old man was diagnosed with scalp angiosarcoma without lymph node or organ metastasis. The patient was treated with 10 courses of tri-weekly paclitaxel therapy, radical radiotherapy (70 Gy), and surgical excision (2 cm margin from the lesion). As no recurrence was identified in one year after surgery, we tried to reconstruct the skin defects with tissue expander. However, we could not perform surgical reconstruction because of the expander infection.

Two years after the initial operation, he suffered from a sudden onset of right-sided hemiplegia, emesis, and disturbance of consciousness. Brain magnetic resonance imaging revealed a 50-mm ring-enhancing lesion in the left frontal lobe and a small amount of air in the lesion site, which was not compatible with invasive angiosarcoma but rather a brain abscess (Figure 1a). Although the brain abscess was initially managed with antibiotics (vancomycin and meropenem), his consciousness gradually worsened after 5 days of conservative treatment. Computed tomography revealed his brain abscess had enlarged, and emergency drainage of the abscess was performed. The pus liberated from the site was yellowish-white and viscous (Figure 1b). Bacterial cultures of the brain abscess specimens revealed an infection with *Finegoldia magna*. His clinical symptoms improved within 24 hours of emergency drainage. At two months after drainage, we performed sequestrectomy of the cranial bone and performed reconstructive surgery using the free rectus abdominis myocutaneous flap to cover the exposed lesion. He achieved incident-free survival without the recurrence of the angiosarcoma or brain abscess for three years.

To our knowledge, the only other case of a cerebral lesion in an angiosarcoma patient was a case with pneumocephalus (*2*). This is the first published case describing a brain abscess in an angiosarcoma patient. Abscess formation could be due to large skin defects and cranial bone necrosis resulting from surgery and radiation. Recently, combination therapy with radiotherapy and taxane significantly prolonged overall

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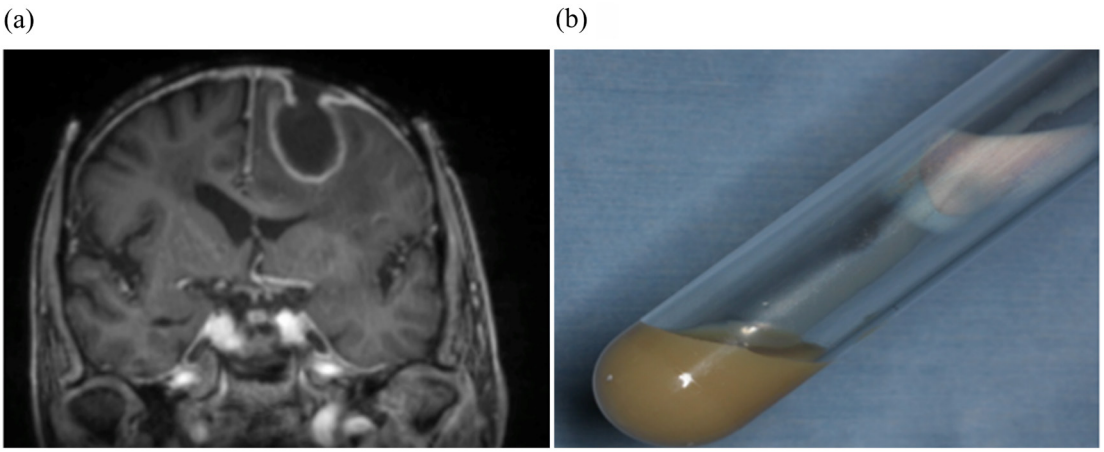

Figure 1. (a) Brain magnetic resonance imaging showing a 50-mm ring-enhancing lesion in the left frontal lobe. (b) Emergency drainage of brain abscess revealed yellowish-white viscous fluid.

survival following surgery (*3*), which may indicate that surgery with large skin defects is not necessary for angiosarcoma. In conclusion, further studies are required to establish a more effective treatment regime for angiosarcoma with fewer side effects.

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