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Original

Identification of a safe and highly specific titanium reagent for patch tests: results from a preliminary clinical trial

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Abstract

Background: Despite the increasing number of titanium patch test studies in Japan, the patch test allergen for titanium has not been standardized. In this study, we tested safety and specificity of titanium reagents to determine the least irritating reagent that attains the highest concentration for healthy subjects.

Methods: This study enrolled 50 healthy volunteers from March 2016 to November 2018. Reagents included 0.1% and 0.5% titanium sulfate, 0.1% titanium chloride, and 0.1% titanium oxide, which were applied on the participants' upper arm for 2 days, and the skin reaction to each reagent was evaluated as positive reaction, irritation, or negative reaction on days 2, 3, and 7 based on the International Contact Dermatitis Research Group criteria. We determined the specificity of the titanium reagents based on the reaction on Day 7.

Results: We detected three false positives for 0.5% titanium chloride and one for the 0.1% titanium sulfate solution. The irritant reaction (IR) frequencies were 14% (7/50) for 0.1% titanium chloride, 6% (3/50) for 0.5% titanium sulfate, 6% (3/50) for 0.1% titanium sulfate, and 2% (1/50) for 0.1% titanium oxide, respectively. The 0.5% titanium chloride formulation showed strong irritant ability and was discontinued after use in 27 patients. We found high specificity in the order of 0.98 for 0.1% titanium oxide, 0.94 for 0.5% titanium sulfate, and 0.92 for 0.1% titanium sulfate, respectively.

Conclusions: Among the ionized reagents, 0.5% titanium sulfate was identified as the most suitable reagent, with low irritation, and a low false-negative rate for patch tests.

Key words：titanium allergy, diagnostic agent, patch test

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Background

Titanium is considered to be non-allergenic, but there have been several reports of cases of metal allergies caused by titanium-containing materials[1–3]. Thus, there is an increasing need for titanium allergy testing and, therefore, a patch test to diagnose titanium allergies is needed, similarly to that used for other metal materials.

Despite the increase in number of titanium patch test studies in Japan, the patch test allergen for titanium has not been standardized. Nakajima reported that an allergen composed of pure titanium powder and vaseline was inadequate, and suggested titanium chloride (0.1%) as a preferable patch test allergen for titanium[4], since titanium oxide does not contain titanium ions or has almost no ionization, and therefore, unlikely cause allergic contact dermatitis by itself. Hosoki et al. performed patch tests with titanium oxide (30% and 10%) and titanium chloride (0.1% and 0.05%) for patients who visited a dental clinic in Tokushima, Japan, and found that 25% and 6.25% of 93 patients who received a titanium dental implant showed positive reactions to 0.1% and 0.05% titanium chloride, but none of them showed a positive reaction neither to 30% nor 10% titanium oxide[5]. In the present study, we used titanium sulfate, titanium chloride, and titanium oxide as patch test reagents. Titanium chloride, titanium oxide, titanium (1% pet.), calcium titanate (10% pet.), titanium nitride (5% pet.), and titanium oxalate decahydrate (5% pet.) are often used in studies as titanium-containing reagents[4–6]. However, we often experience irritation reactions (IRs) to titanium chloride, as shown in this study. Furthermore, titanium oxide, calcium titanate, titanium nitride, and titanium oxalate decahydrate do not ionize easily. Therefore, we added titanium sulfate as a test reagent in this study.

In a previous study, we described the challenges in ascertaining titanium sensitivity due to the high percentage of false positives (?+) or IR findings with a titanium reagent[7], which can be potentially attributed to the strong acids, such as sulfuric or hydrochloric acid, that are used to convert metals to ions in the reagent. However, these reactions have often been judged as positive reactions in patients with delicate skin. Thus, it is important for titanium regents to be safe and have low irritability.

In this study, we tested safety and specificity of patch test with 0.1% and 0.5% titanium sulfate, 0.1% and 0.5% titanium chloride, and 0.1% titanium oxide to determine the reagent and its concentration that was non-irritating and minimum rate of false negatives in healthy subjects. We also added titanium oxide, since it is widely used and the only one commercially available titanium reagent.

Materials and Methods

2. 1. Ethical approval of the study protocol

The study protocol was approved by the clinical research ethics review committee of the authors' university (approval no. C-72), and was conducted in accordance with the Declaration of Helsinki (1964) and its later amendments.

2. 2. Participants

Fifty adult volunteers (28 males and 22 females; average age 37.2 years, range 20–75 years), who provided written informed consent for study participation were enrolled from March 2016 to November 2018. All subjects had no history of allergies in the medical interview, and were assumed as non-allergic to titanium.

2. 3. Patch test

Titanium reagents, either 0.1% or 0.5% titanium sulfate and 0.1% titanium chloride, were prepared by diluting 30% titanium sulfate and 16–17% titanium chloride (Wako Pure Chemical Co., Ltd, Osaka, Japan), respectively, with distilled water prepared in our institution. We purchased 0.1% titanium oxide (allergEAZE®) from Brial Allergy GmbH, Germany. The allergens were applied to the upper arm by using a Patch Tester (Torii Pharmaceutical Co., Ltd, Tokyo, Japan), and the sheets were removed after 2 days. Skin reactions were classified on days 2, 3, and 7 by using the International Contact Dermatitis Research Group (ICDRG) crite-

ria. The positive reaction, IR, and specificity to 0.1% and 0.5% titanium sulfate solution, 0.1% titanium chloride solution, and oxidized titanium on Day 7 after the test were evaluated according to the ICDRG recommendation of late reading at 7 days after the patch test application to identify positive reactions in the case of "late reactors"[8].

Results

3. 1. Judgment for each titanium reagent

Tables 1 and 2 show the results of the patch test for 0.1% and 0.5% titanium sulfate solution, 0.5% and 0.1% titanium chloride solution, and oxidized titanium on Day 7 after the patch application. We found three false positives (11.1%) for 0.5% titanium chloride and one (2.0%) for 0.1% titanium sulfate solutions (Table 1). The use of 0.5% titanium chloride was discontinued after the 27th participant because of the high frequency of irritation (16/27, 59%). The IR frequency was 14% (7/50) for 0.1% titanium chloride, 6% (3/50) for 0.5% titanium sulfate, 6% (3/50) for 0.1% titanium sulfate, and 2% (1/50) for 0.1% titanium oxide, respectively (Table 2).

3. 2. Specificity of each titanium reagent

In descending order, the specificity was 0.98 for 0.1% titanium oxide, 0.94 for 0.5% titanium sulfate, 0.92 for 0.1% titanium sulfate, 0.86 for 0.1% titanium chloride, and 0.30, for 0.5% titanium chloride, respectively (Table 3).

Discussion

Titanium has become an indispensable agent in the medical and dental fields. However, titanium allergies have been increasingly reported, including spontaneous rapid exfoliation of the implant or repeated failures without any infection or overload risk factors. A titanium allergy is suspected as the cause of the implant failure or dermatitis after dental implantation and must be diagnosed by a patch test. However, there is no standard titanium reagent for use in patch tests in Japan. Thus, different forms and concentrations of titanium compounds are used at individual facilities. Therefore, it is important to standardize and unify the reagents not only to make a proper diagnosis for individual patients, but also to know the prevalence of titanium allergy among patients and/or society. This study was conducted to determine the safety and high specificity of ionized titanium reagent in order to accurately reveal the current status of titanium allergies in Japan.

In accordance with the mechanism of a type Ⅳ allergy, ionized metals can bond with native proteins to form haptenic antigens, or they can trigger degranulation of mast cells and basophils to thereby produce a type Ⅰ or Ⅳ hypersensitivity reaction[9, 10]. Therefore, it is important that the titanium reagent is ionized or easily ionized in the skin patch test. A few are authors suggested that 0.1% and 0.2% titanium sulfate and 0.1% and 0.2% titanium chloride solutions are appropriate reagents for the skin-patch test, and could be a valuable alternative to the titanium oxide formulation that is widely used in patch tests[4, 5, 11]. However, there are no clear data of these reagents obtained in a detailed study.

Tests for a titanium allergy in dentistry are conducted at two time points. One is undertaken for suspected post-treatment allergic reactions, and the other is for the preoperative examination (e.g., before an implantation). A patch test after

Table 1 Titanium reagents and breakdown of patch test judgment

Reagents	Negative (%)	False positive (%)	Irritant reaction (%)
0.5% titanium sulfate solution	47 (94.0)	0 (0.0)	3 (6.0)
0.1% titanium sulfate solution	46 (92.0)	1 (2.0)	3 (6.0)
0.5% titanium chloride solution	8 (29.6)	3 (11.1)	16 (59.2)
0.1% titanium chloride solution	43 (86.0)	0 (0)	7 (14.0)
0.1% titanium oxide	49 (94.0)	0 (0)	1 (2.0)
Nothing	49 (94.0)	0 (0)	1 (2.0)

Table 2 The results of the patch test for all cases on the 7th day

Case No.	0.5% titanium sulfate solution	0.1% titanium sulfate solution	0.5% titanium chloride solution	0.1% titanium chloride solution	0.1% titanium oxide	Nothing※
1	−	−	IR	IR	−	−
2	−	−	−	−	−	−
3	IR	IR	IR	−	−	−
4	−	−	−	−	−	−
5	−	−	IR	−	−	−
6	−	−	−	−	−	−
7	−	−	IR	−	−	−
8	−	−	IR	IR	−	−
9	−	−	IR	−	−	−
10	−	−	−	−	−	−
11	−	−	?+	−	−	−
12	−	−	IR	−	−	−
13	−	−	IR	−	−	−
14	−	−	IR	IR	−	−
15	−	−	−	−	−	−
16	−	−	IR	IR	−	−
17	−	−	−	−	−	−
18	IR	IR	?+	IR	−	−
19	−	?+	?+	−	−	−
20	−	−	IR	−	−	−
21	−	−	IR	−	−	−
22	−	−	−	−	−	−
23	−	−	IR	−	−	−
24	−	−	IR	−	−	−
25	−	−	IR	−	−	−
26	−	−	IR	−	−	−
27	−	−	−	−	−	−
28	−	−	*	−	−	−
29	−	−	*	−	−	−
30	−	−	*	−	−	−
31	−	−	*	−	−	−
32	−	−	*	−	−	−
33	−	−	*	−	−	−
34	−	−	*	−	−	−
35	−	−	*	−	−	−
36	−	−	*	−	−	−
37	−	−	*	−	−	−
38	−	−	*	−	−	−
39	−	−	*	−	−	−
40	IR	IR	*	IR	IR	IR
41	−	−	*	−	−	−
42	−	−	*	−	−	−
43	−	−	*	−	−	−
44	−	−	*	IR	−	−
45	−	−	*	−	−	−
46	−	−	*	−	−	−
47	−	−	*	−	−	−
48	−	−	*	−	−	−
49	−	−	*	−	−	−
50	−	−	*	−	−	−

※ : Nothing on the sheet.

* : The use of 0.5% titanium chloride was discontinued after the 27th participant.

− : Negative, ?+ : False Positive, IR : Irritant reaction

Table 3 Specificity of each titanium reagent

Reagents	Specificity
0.5% titanium sulfate solution	0.94
0.1% titanium sulfate solution	0.92
0.5% titanium chloride solution	0.30
0.1% titanium chloride solution	0.86
0.1% titanium oxide	0.98

the appearance of allergic symptoms is usually undertaken to identify the allergen. Allergy to titanium in the implant may cause peri-implantitis and allergic dermatitis. Since implant treatment is time consuming and expensive, implant failures are a large burden on the patient than those of the other treatments. Although patch tests can induce allergies by itself, the risk of titanium allergies should be evaluated preoperatively to prevent implant survival or dropout. If an allergic reaction to titanium is detected during a preoperative examination, prosthetic appliances such as bridges and dentures made of materials other than titanium could be used as alternatives to implants.

Based on various opinions[2, 5, 10, 11] that have been reported in the literature, we ascertained a need to identify the form and concentration of titanium reagents that could accurately generate a positive reaction without sensitizing an allergy-negative individual to titanium. Although there had been no report so far, we added titanium sulfate as an alternative reagent to titanium chloride and titanium oxide which are widely used in clinical practice. Titanium sulfate, at a concentration in the range of 0.1-0.5%, appears to be the most suitable test reagent, with good specificity ad a non-problematic degree of skin irritation. Based on clinical data, the IR percentage of both 0.1% and 0.5% titanium sulfate was 6%, which was not as low as that of silver, or as high as that of gold or zinc (data not shown). Misdiagnosis of IR by the titanium sulfate reagents ought to be avoided by understanding IR and appropriately reading the patch test.

In our results, the specificity of titanium chloride is lower than that of the other test reagents. The reason for such difference remains unknown. Since completely free titanium ions derived from titanium chloride and titanium sulfate in aqueous solution should be the same, titanium ion associated with less than four chloride ions or non-ionized titanium chloride might cause skin irritation for a certain type of subjects. Difference in degree of hydroxide formation by titanium chloride or titanium sulfate dissolved in water[12] may be another reason of the irritation.

Patch test reagents ought to possess not only specificity but also sensitivity. In this study, sensitivity was not examined due to difficulty of recruiting patients with clinically clear titanium allergy. However, an increase of titanium concentration to up to 0.5% preserving the low false-positive reaction should be advantageous to achieve high sensitivity for real titanium allergy.

Conclusions

The 0.5% titanium sulfate appears to be the most suitable as a reagent for skin patch test, in that it shows high specificity and less irritation. Overall clinical suitability of titanium sulfate including sensitivity and stability in aqueous solution should be validated by testing subjects with authentic titanium allergy.

Ethical Approval and Consent to participate

The study protocol was approved by the clinical research ethics review committee of the authors' university (approval no. C-72), and was conducted in accordance with the Declaration of Helsinki (1964) and its later amendments. We described the purpose of the study to patients, and the data obtained were anonymized. Written informed consent was obtained from patients for the publication of this report and any data.

Consent for publication

Written consent to publish was obtained from all participants.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

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Author's contributions

The study was conducted and designed by MK, KI, MT and HK. The results were analyzed and interpreted by HO, MH and MK. MK wrote the manuscript. MH, MT and HS reviewed and edited the manuscript. All authors read and approved the final manuscript.

Abbreviations

IR : Irritant reaction

ICDRG : International Contact Dermatitis Research Group

Trial registration

UMIN, UMIN000021240. Registered 29 February 2016, https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000024350

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