

Higher Prevalence of Nickel and Palladium Hypersensitivity in Patients with Ulcerative Colitis

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Keywords

Ulcerative colitis · Inflammation · Delayed-type hypersensitivity · Lymphocyte transformation test

Abstract

Background: The etiology of ulcerative colitis (UC) remains elusive even though many genetic and environmental pathogenic factors have been reported. Aberrant inflammatory responses mediated by specific subsets of T cells have been observed in ulcerative lesions of UC patients. **Objectives:** To elucidate the involvement of a delayed-type hypersensitivity reaction in UC, we focused on dental metal hypersensitivity, a T cell-mediated, delayed-type allergic reaction that causes oral contact mucositis and systemic cutaneous inflammation. **Method:** We recruited 65 Japanese UC patients and 22 healthy controls (HC) and used the in vitro lymphocyte stimulation test to quantify their sensitivity to zinc, gold, nickel, and palladium – the metals that have been widely used in dentistry. All subjects were users of metallic dental implants and/or prostheses containing zinc, gold, nickel, and/or palladium as major constituents. **Results:** Sixty percent of the UC patients were hypersensitive to at least one metal species, whereas 32% of the HC were hypersensitive to only a single metal species. The overall incidence of

metal hypersensitivity was significantly higher for UC patients than for HC. Furthermore, a significantly greater proportion of UC patients were hypersensitive to nickel or palladium. The severity of the sensitivity to nickel and palladium was also significantly greater for UC patients than for HC. **Conclusions:** This pilot study demonstrates that UC patients have a significantly higher incidence of hypersensitivity to nickel and palladium, suggesting the possible involvement of dental metal hypersensitivity in UC pathogenesis.

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Introduction

Ulcerative colitis (UC) is characterized by chronic inflammation of the colonic mucosa with alternating periods of exacerbation and remission. Several contributing factors to the mucosal inflammation have been suspected, but the etiology remains elusive. In ~30% of UC patients, colonic symptoms occur in conjunction with a wide variety of extraintestinal manifestations including erythema nodosum of the skin, which is presumed to be caused by a delayed-type hypersensitivity (DTH) reaction [1]. No-

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tably, the colonic disease activity of UC changes in parallel with the erythema nodosum activity [1]. These aspects of UC suggest that the DTH reaction is a shared pathogenic mechanism between the colonic inflammation and erythema nodosum. Nevertheless, few reports have focused on how the DTH reaction might be associated with UC.

To elucidate the involvement of DTH in the mucosal inflammation in UC, we focused on hypersensitivity to dental metals, a T cell-mediated DTH reaction that causes oral contact mucositis and systemic dermatitis [2, 3]. Hypersensitivity to dental metals strongly correlates with cutaneous inflammatory lesions such as palmoplantar pustulosis [4], lichen planus [5], and dyshidrotic eczema [6]. Metal hypersensitivity is also closely associated with a subset of inflammatory autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome [7]. In UC, using the European Standard Series of patch test allergens, Engkilde et al. [8] found no significant correlation between patch test results and UC symptoms. This allergen series, however, includes only cobalt and nickel as metal allergens, potentially underestimating the association between metal hypersensitivity and UC. We hypothesized that trace amounts of metal cations persistently released from dental alloys could elicit a DTH reaction in the colonic mucosa and induce the chronic inflammation observed in UC patients. We thus conducted a case-control study to examine the prevalence of hypersensitivity to zinc, gold, nickel, and palladium, the metals that have been commonly used for dental implants and prostheses, in UC.

Materials and Methods

Subjects

A total of 65 Japanese outpatients were enrolled (34 males, 31 females; age range, 18–69 years; mean age [SD], 40.0 [10.9] years). Each patient was first diagnosed with UC at the age of 14–68 (mean age [SD], 33.2 [11.5] years; duration range, 0–30 years; mean duration [SD], 7.2 [7.3] years) and transferred to the gastroenterology department of Takanawa Clinic in the period from October 2017 through July 2018. All patients were classified into the ulcerative proctitis subtype based on colonoscopic observation. Patients >70 years of age or those who were pregnant were excluded. A total of 22 healthy controls (HC) were recruited (7 males, 15 females; ages 12–70 years; mean age [SD], 48.9 [13.7] years). Individuals were excluded from the HC group if they had current or previous UC or other major gastrointestinal diseases, or inflammatory diseases. Individuals with a history of hypersensitivity to allergens other than metals were also excluded. All subjects were users of metallic dental implants and/or prostheses containing zinc, gold, nickel, and/or palladium as major constituents.

Lymphocyte Transformation Test

We employed the lymphocyte transformation test (LTT) to quantitatively evaluate sensitivity to metal cations [9, 10]. LTT was outsourced to SRL, Inc. (Tokyo, Japan). Briefly, lymphocytes were isolated from heparinized peripheral blood by density gradient centrifugation using Ficoll-Conray media (Lymphosepar I; Immuno-Biological Laboratories) and resuspended at $1 \times 10^6/\text{mL}$ in RPMI1640 medium supplemented with 20% autologous plasma. The 200 μL aliquots (2×10^5 cells) were cultured in triplicate in a 96-well microplate with a 5-fold dilution series (1:50–1:156,250) of 2% ZnCl_2 , 0.2% HAuCl_4 , 5% NiSO_4 , or 1% PdCl_2 or with phytohemagglutinin (PHA; 7 $\mu\text{g}/\text{mL}$) at 37 °C for 72 h in a 5% CO_2 incubator. Proliferation activity was measured by the addition of (Methyl- ^3H)thymidine (10.57 kBq/well; Moravek Inc.) for 18 h, followed by harvesting of cells on filters. Radioactivity retained on the filters was measured using a scintillation counter (PerkinElmer Inc.). Any subject with a stimulation index (SI) of 180 or more was regarded as a hypersensitive person: $\text{SI} = 100 \times (\text{the amount [cpm] of } ^3\text{H-thymidine incorporated into peripheral blood lymphocytes of a subject during stimulation with a metal species/the amount [cpm] with no stimulation})$. PHA was used as a positive control.

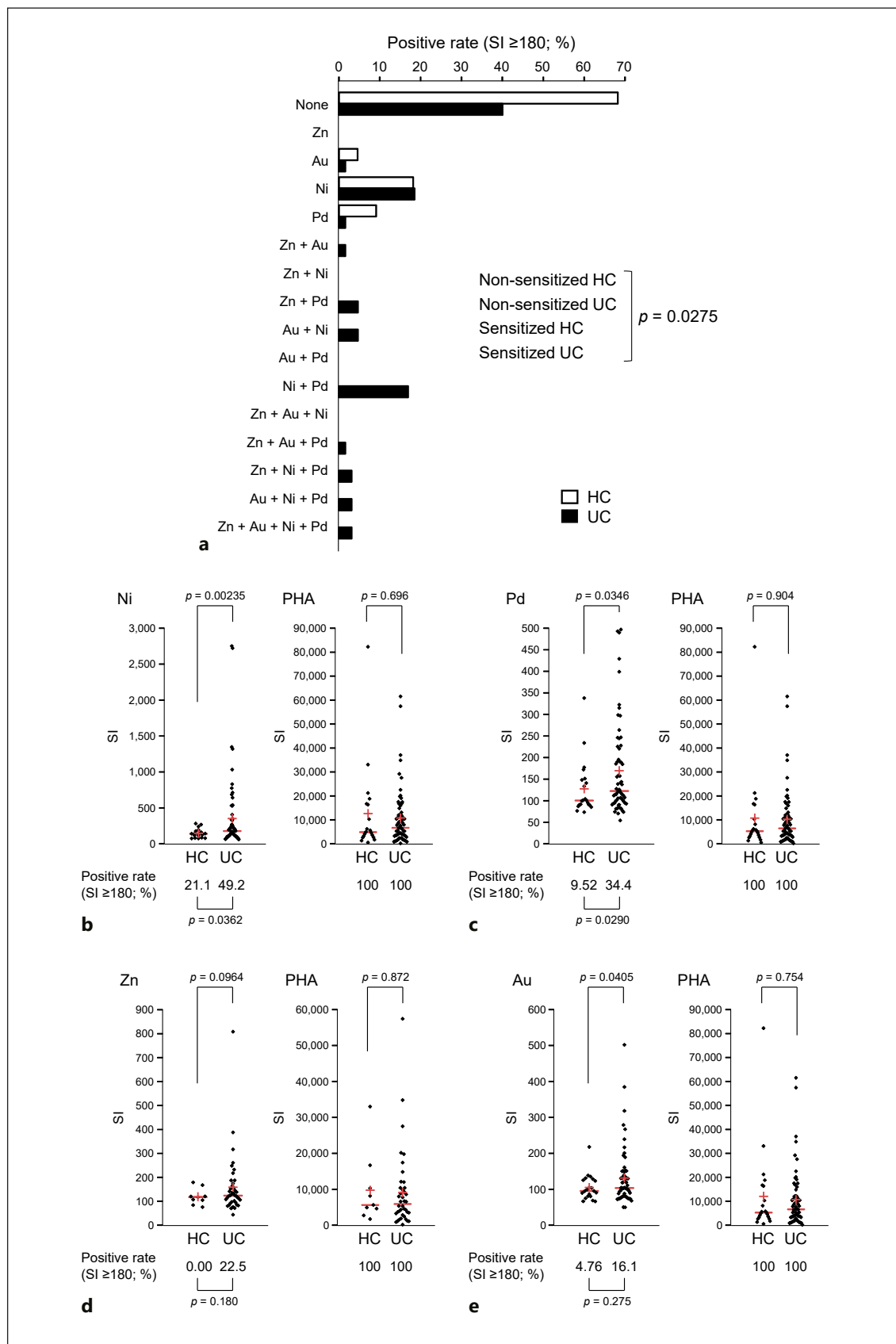
Statistical Analysis

Two-tailed Fisher's exact test and two-tailed Welch's *t* test were performed using Prism8 (GraphPad Software). No outliers were taken into account, and all collected data were subjected to statistical analysis.

Results

The UC patients and HC individuals were subjected to the LTT for zinc, gold, nickel, and palladium. The majority of the HC (68.2%) were negative for sensitization to any metals examined, and the rest (31.8%) were positive for sensitization to only a single metal species (Fig. 1a). In contrast, 60.0% of the UC patients were hypersensitive to at least one metal species, and 38.5% of the patients were hypersensitive to more than one species. Two UC patients were sensitized to all four species examined. Overall, the incidence of metal hypersensitivity was significantly higher for UC patients than for HC ($p = 0.0275$, two-tailed Fisher's exact test; odds ratio = 3.21, 95% confidence interval: 1.15–8.96; Fig. 1a).

In line with these results, a significantly greater proportion of UC patients were hypersensitive to nickel ($p = 0.0362$, two-tailed Fisher's exact test; odds ratio = 3.64, 95% confidence interval: 1.09–12.14; Fig. 1b) or palladium ($p = 0.0290$, two-tailed Fisher's exact test; odds ratio = 4.98, 95% confidence interval: 1.06–23.34; Fig. 1c) as compared with the HC group. In addition, the mean SI values for UC patients with respect to nickel and palladium were significantly higher than those for HC (nickel: $p = 0.00235$, two-tailed Welch's *t* test, Fig. 1b; palladium: $p = 0.0346$, two-tailed



(For legend see next page.)

Welch's *t* test, Fig. 1c). However, there was no significant difference in the incidence of zinc or gold hypersensitivity between UC patients and HC, although the mean SI value for gold was significantly higher for the UC group than for HC ($p = 0.0405$, two-tailed Welch's *t* test, Fig. 1d, e).

Discussion

All subjects recruited in this study were regular users of metallic dental implants and/or prostheses containing zinc, gold, nickel, and/or palladium as major constituents. Nickel has long been known to be the major cause of metal hypersensitivity [11–13]. Palladium hypersensitivity has become an issue of growing concern owing to its increased use in industry, jewelry, and dentistry over the last few decades [14]. In a recent European multicenter study, the prevalence of palladium- or nickel-sensitive individuals was estimated at 24.3 and 25.2%, respectively, and, notably, sensitization to palladium was significantly associated with exposure to dental alloys [15]. Furthermore, Kitagawa et al. [16] reported that 22.5 and 14.8% of the Japanese dental patients were sensitization-positive for nickel and palladium, respectively, based on results from the patch test, and 67.9% of the patients who were hypersensitive to any metal species were found to use dental alloys containing the respective sensitizing metal(s).

Colonic inflammation of UC is known to be caused by dysregulated immune responses mediated by specific subsets of T cells: Th1, Th2, Th17, and Th9 [17]. Various kinds of pro- and anti-inflammatory cytokines related to these T-cell subsets are aberrantly increased or decreased in the inflamed mucosa and serum of UC patients [18]. A large body of evidence indicates that similar activation of

the T-cell subsets has also been observed in patients with nickel or palladium hypersensitivity [19]. Peripheral blood mononuclear cells from palladium- or nickel-sensitive individuals show mixed Th1/Th2 cytokine production in response to palladium or nickel exposure, respectively [20]. Interestingly, Cristaudo et al. [21] reported that dental prostheses are the major source of palladium that can induce palladium-specific T-cell responses and IFN- γ production underlying palladium hypersensitivity. In addition, dermal infiltration of Th17 cells and the Th17-related cytokine levels are markedly elevated in nickel-challenged skin biopsies from nickel-sensitive patients [22, 23]. IL-17 acts on epithelial cells to induce the production of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α [24]. Similarly, the number of Th9 cells and expression of IL-9 and PU.1, the master transcription factor for Th9, are significantly increased in the dermal infiltrate of skin biopsies from patients with nickel hypersensitivity [25, 26]. IL-9 promotes colonic inflammation by rendering inflammatory cells resistant to apoptosis and impairs the intestinal barrier function by inhibiting wound healing of the colonic epithelium [27]. Collectively, chronic exposure to nickel or palladium cations may provoke the activation of specific T-cell subtypes similar to that observed in UC. Replacement of dental alloys with non-metal materials, as well as dietary control of nickel and palladium intake, may thus enhance the therapeutic effect of existing pharmacotherapy for UC patients. In this context, it is interesting to note that a low-nickel diet clearly alleviates the gastrointestinal symptoms of patients with irritable bowel syndrome [28], a disease that involves low-grade mucosal inflammation and aberrant T-cell activation similar to those in UC [29–32].

This pilot study suggests that hypersensitivity reaction to nickel and palladium may underlie aberrant immune activation in the UC mucosa. However, the small sample size may limit its conclusions and generalizability. Further studies with larger cohorts and from a mechanistic perspective will be required to firmly establish whether a causative link exists between hypersensitivity to orally derived metals and UC pathogenesis.

Conclusion

This study demonstrates that patients with UC have a significantly higher prevalence of hypersensitivity to nickel and palladium, suggesting that hypersensitivity reaction to nickel and palladium may underlie the mucosal inflammation in UC.

Fig. 1. UC patients are more sensitive to nickel and palladium than are healthy individuals. **a** The incidence of hypersensitivity to zinc, gold, nickel, and/or palladium (SI ≥ 180) in healthy controls (HC, open bar) and patients (UC, closed bar). Subjects in both groups were regular users of metallic dental implants and/or prostheses containing zinc, gold, nickel, and/or palladium as major constituents. **b–e** Distribution of SI values for HC and UC patients with respect to nickel (Ni, **b**), palladium (Pd, **c**), zinc (Zn, **d**), and gold (Au, **e**). Each red horizontal line and red cross corresponds to the median and the mean, respectively. An overall LTT-positive (SI ≥ 180) rate in each metal species is shown below the bee swarm. Phytohemagglutinin (PHA) was used as a positive control in each test. All subjects were positive for PHA, and there was no significant difference in the mean SI values between HC and UC patients. The data were statistically analyzed with two-tailed Fisher's exact test (**a–e**) and two-tailed Welch's *t* test (**b–e**). *p* values < 0.05 were regarded as statistically significant.

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Statement of Ethics

This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All procedures were approved by the Ethics Committees of the Takanawa Clinic (approval number: 2018-2). A signed informed consent form was obtained from each participant prior to inclusion in this study. All experimental procedures and data analyses were conducted by investigators who were blinded to the subjects' clinical information using a de-identified dataset.

Disclosure Statement

Y.K., Y.S., K.K., M.M., and K.A. are employees of Takanawa Clinic. T.A. and T.N. have advisory roles in conducting clinical research in Takanawa Clinic and receive advisory fees from Takanawa Clinic.

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Author Contributions

Y.K., Y.S., K.K., M.M., and K.A. contributed to the conception and design of the study, contributed to data acquisition, analysis, and interpretation, and critically revised the manuscript for important intellectual content. T.A. contributed to the conception and design of the study and critically revised the manuscript for important intellectual content. T.N. contributed to the conception and design of the study, contributed to data analysis and interpretation, and drafted the manuscript. All authors gave final approval of the submitted version of the manuscript and agree to be accountable for all aspects of the work.

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