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Manganese Oxidation State as a Cause of Irritant Patch Test Reactions

Laurie Shallcross, MD^{*}, Simon Ritchie, MD^{*}, Erin Harberts, BA^{*,†}, Antonella Tammaro, MD[‡], Joanna Gaitens, PhD[§], and Anthony A. Gaspari, MD^{*,†}

^{*}Department of Dermatology, University of Maryland School of Medicine, Baltimore MD

[†]Department of Microbiology/Immunology, University of Maryland School of Medicine, Baltimore MD

[‡]Department of Dermatology, La Sapienza University, Rome, Italy

[§]Department of Medicine, University of Maryland School of Medicine, Baltimore MD.

Abstract

Background—Manganese chloride (MnCl₂) 2.5% is included in the extended metals patch test series to evaluate patients for contact hypersensitivity to this metal salt.

Objectives—The objective of this study was to prospectively determine the rate of allergic and irritant patch test reactions to MnCl₂ (Mn(II)), Mn₂O₃ (Mn(III)), and KMnO₄ (Mn(VII)) in a cohort of patients undergoing patch testing.

Methods—Fifty-eight patients were patch tested with MnCl₂, Mn₂O₃, and KMnO₄, each at 2.5% in petrolatum. Patch readings were taken at 48, and 72 or 96 hours, and scored using standard methods. Cultured monolayers of keratinocytes (KCs) were exposed to MnCl₂, Mn₂O₃, and KMnO₄ in aqueous culture medium, and cell survival and cytokine release were studied.

Conclusions—MnCl₂ caused irritant patch test reactions in 41% of the cohort, whereas Mn₂O₃ and KMnO₄ caused a significantly lower rate of irritant reactions (both 3%). No allergic morphologies were observed. Similarly, in cultured KC monolayers, only MnCl₂ was cytotoxic to KC and induced tumor necrosis factor α release.

The oxidation state of manganese used for patch testing affects the irritancy of this metal salt, as Mn(II) caused an unacceptably high rate of irritant reactions in a cohort of patients. In vitro studies confirmed these clinical data, as only Mn(II) was cytotoxic to cultured monolayers of KC.

Manganese (Mn) is a ubiquitous environmental element, making up approximately 0.1% of the Earth's crust as a hard, brittle metal.¹ Multiple oxidation states ranging from −3 to +7 exist, with the most common states being +2, +3, and +7 (Mn(II), Mn(III), and Mn(VII), respectively).² Most exposure to manganese occurs through dietary intake of the divalent and tetravalent forms.¹ Once consumed, manganese functions as a cofactor in multiple

enzymatic reactions and participates in bone growth, immune function, blood coagulation, reproduction, and digestion.³ Currently, no cases of manganese deficiency have been reported, but there are numerous examples of manganese toxicity from increased exposure. Welders experience higher rates of a Parkinson disease-like illness, which is attributed to the high levels of manganese in welding consumables.⁴ In the general population, proximity to industrial plants where manganese compounds are being used may lead to increased exposure.⁵ Because of an increase in orthodontic procedures, hip replacements, and body art, there is a growing population of individuals that may be at higher risk of developing Mn allergy, or even toxicity.

Despite the increase in use of implantable devices using manganese, there are few case reports of patients with allergic contact dermatitis attributable to manganese, and these case reports are still considered questionable.⁶ A study assessing allergic reactions to metals commonly used in orthodontic implants examined the rates of reactions to manganese (II) chloride (MnCl_2) before and after orthodontic implantation and found that there was no significant difference in positive reactions after appliance placement.⁷ Another study looked at sensitivity to metals after failed metal on metal arthroplasty and found that 1 of 16 subjects exhibited patch test reactivity to manganese after the failed arthroplasty. However, in the same study, 4 of 16 patients exhibited reactivity to manganese utilizing a lymphocyte transformation test.⁸

Currently, the extended metal series (Chemotechnique) is utilized to comprehensively evaluate a patient for allergy to “unusual” metals (above and beyond nickel, cobalt, chromates, or gold), either in an occupational setting or in the setting of a metal-containing medical device (such as a dental, orthopedic, or intravascular implant). Despite the use of the extended metal series to evaluate patients for metal allergy, many of the metal salts included in this series lack comprehensive evaluations (epidemiologic data) to define optimal (nonirritating) patch test concentrations. If a patient with an implanted device containing manganese is suspected to have an allergy to manganese, the current approach is to utilize MnCl_2 (Mn^{2+} oxidation state) at a 2.5% concentration in petrolatum. As manganese exists in multiple oxidation states,² it is possible that only a certain oxidation state or states may be responsible for eliciting allergy or irritation, whereas others may have no effect; this concept has yet to be fully studied.

The purpose of this study was to determine the rate of allergic and irritant reactions to manganese of 3 different oxidation states using standard patch test methods. The demographics of the patients who reacted to each oxidation state of manganese were compared with each other to attempt to determine what patients may be at higher risk for these reactions.

METHODS

Patch Testing

This study was approved by the institutional review board of the University of Maryland. Fifty-eight patients who were being evaluated by the Department of Dermatology of the University of Maryland for suspected contact dermatitis were randomly selected to

participate in the study. The characteristics of our study population are summarized in Table 1. After providing informed consent, the patients were patch tested to Mn(II), Mn(III), and Mn(VII) under 8-mm Finn chambers in addition to the North American Contact Dermatitis Group series, regardless of whether manganese was suspected as a possible allergen. Approximately 20 mg of each chemical was dispensed on each Finn chamber. Mn(II) was tested by using manganese(II) chloride (MnCl_2), which was acquired from Chemotechnique, a Canadian laboratory. Mn(III) was tested by using manganese(III) oxide (Mn_2O_3) and was acquired from F.I.R.M.A (Milan, Italy). Mn(VII) was tested by applying potassium permanganate (KMnO_4), F.I.R.M.A. These 3 Mn salts were compounded in petrolatum to achieve a concentration of 2.5%. The patches were securely adhered to the patients' backs (Finn chambers attached to Scanpore tape and bolstered with tape) and left under occlusion for 48 hours before removal. The patients were instructed to avoid showering, exercising, or any activity that would result in excess moisture between the time of patch placement and removal. Once the tape and Finn chambers were removed, a grid was marked on the patients' backs detailing the location of each allergen, and photographs were taken to allow easy identification at a subsequent read 72 hours after patch placement and, if necessary, at a time further in the future. Each patient also completed a questionnaire relating to demographics and their history of contact dermatitis and atopic dermatitis.

Patch test readings were taken at 48 hours, 72 hours, and, in some cases, at 96 hours after patch application. The reactions were scored and recorded according to the International Contact Dermatitis Research Group system.⁹

In Vitro Studies of KC

Primary human keratinocytes (KCs) derived from human fore-skins, FSKCs, and a human KC cell line, HaCaT, were studied.¹⁰ These cell lines were incubated with 4 different concentrations of Mn(II), Mn(III), and Mn(VII) (provided by F.I.R.M.A) (0.386, 0.772, 1.544, and 3.088 mM) to ascertain their effects on cell survival after 24 hours. The 3 Mn formulations were powders and were soluble in aqueous culture medium and diluted at the appropriate concentrations to result in the listed molarities using Dulbecco modified Eagle medium supplemented with fetal calf serum and antibiotics.¹⁰ The compounds used for each manganese preparation were identical to those used for the patch testing portion of this experiment. Controls were also established by incubating FSKCs and HaCaT cells in medium alone for 24 hours. After 24 hours, the supernatants of all incubations were removed. Following the removal of the supernatant, thiazole blue tetrazolium bromide was added to each of the incubations (MTT assay).¹¹ After 4 hours of exposure to MTT, the optical density of the cell lines was measured to determine cell survival.¹¹

Cell-free supernatants from the medium alone and the 1.544-mM concentration of all 3 Mn oxidation states were removed at 24 hours. The amount of tumor necrosis factor α (TNF- α), interleukin 1 (IL-1), and IL-6 in each supernatant was measured using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Both the cytotoxicity and the TNF- α release assays were repeated at least twice and found to be reproducible with either primary KC or HaCaT cell lines.

Statistical Analyses

Instat and Prism software programs were utilized to identify statistically significant differences between KCs treated with different oxidation states (either survival or TNF- α release; analysis of variance [ANOVA]); $P < 0.05$ was considered significant. This same test was used to study rate of irritant patch test reactions. Bivariate statistical analysis of the demographic data was performed; $P < 0.05$ was considered statistically significant.

RESULTS

Patch Testing With Mn(II) Results in a Higher Rate of Irritant Reactions Than Does Mn(III) or Mn(VII)

All 58 patients completed the study, and there were no reactions with allergic morphology noted in any patient. Of the 58 patients tested, 24 (41% of the patch-tested population) demonstrated an irritant reaction to Mn(II), and 2 patients (3% of the patch-tested population) had an irritant reaction to both Mn(III) and Mn(VII), but neither of these patients experienced any reaction to the Mn(II) preparation. This remarkable difference in rate of irritant reactions between Mn(II) and Mn(III) or Mn(VII) is highly significant ($P < 0.05$). The remaining 32 patients demonstrated no reaction to any Mn preparations (Fig. 1A). In Figure 1B, the typical morphologic appearance of patch test reactions to Mn(II) in 3 different patients in our study is depicted. These reactions had the appearance of irritant reactions, exhibiting patchy macular erythema with pustules and rim reactions, partially following the outline of the Finn chamber, but without infiltration. These reactions were most notable at the 48-hour reading and diminished or disappeared by 72 to 96 hours, consistent with an irritant reaction (not shown). Interestingly, 2 patients exhibited irritant reactions to Mn(III) and Mn(VII), but not to Mn(II). In both patients, these irritant reactions were pustular reactions noted at 48 hours, which resolved by 72 to 96 hours. Both patients were white males in their 50s; 1 patient had a history of atopic dermatitis and was being evaluated for hand dermatitis and in parallel exhibited a 1+ reaction to carba mix, which was thought to be clinically relevant (not shown), but no other reactions to any other metals. The other patient was being evaluated for an adverse reaction to dental implants, and other than the irritant reactions to Mn(III) and (VII), he exhibited no reactions to patch tests in either the North American Screening Series or the extended metal series (not shown). The pattern of reactivity exhibited by these 2 patients suggests that Mn(III) and (VII) share in common irritant properties that are distinct from that of Mn(II), because they are exclusive. That is, patients who exhibited irritation from Mn(II) ($n = 24$) did not react to Mn(III) or (VII). Patients who exhibited irritation from Mn(III) and Mn(VII) were much less common ($n = 2$), but did not exhibit irritation from Mn(II).

Bivariate analysis revealed that Mn(II) irritant reactions were more frequent in whites ($P = 0.03$, statistically significant) than in other races and those with irritant reactions to other metals such as cobalt ($P = 0.007$, statistically significant). Patient age, history of atopy, smoking status, gender, and suspected metal allergy were not correlated with irritant reactions (Table 2).

In Vitro Studies of Mn(II), Mn(III), and Mn(VII) Reveal That Only Mn(II) Is Toxic to Human KCs and Induces TNF- α Release

The in vitro analysis of KC survival demonstrated that both primary human FSKC (Fig. 2A) and HaCaT cell (Fig. 2B) lines exposed to Mn(II) experienced decreased survival compared with those exposed to Mn(III), Mn(VII), or culture medium alone. Enzyme-linked immunosorbent assay analysis of the cell-free culture supernatants demonstrated that the second highest concentration of Mn(II) (1.544 mM) induced significantly greater TNF- α release compared with Mn(III) and Mn(VII) at the same concentration (Fig. 3), as well as culture medium alone. We also assayed for the proinflammatory cytokines IL-1 and IL-6, but we were not able to detect these cytokines in medium alone or in medium containing any of the 3 Mn salts (data not shown).

Thus, correlating our in vitro and in vivo data, we conclude that Mn(II) irritant patch test reactions may be dependent on TNF- α release by KCs and are common ($n = 24$ patients); in contrast, irritant patch test reactions to Mn(III) and Mn(VII) do not appear to be dependent on inducing TNF- α release from KCs and are less common ($n = 2$ patients). Instead, these 2 oxidation states of Mn (III) and (VII) may induce other mediators of inflammation from epidermal KCs that have not yet been identified.

DISCUSSION

In this study, we demonstrated that irritant reactions to Mn(II) at the standard concentration for patch testing (MnCl₂ at a concentration of 2.5% in petrolatum) are common (41%) in our patch-tested population (58 patients) and were much more common than those induced by Mn(III) or Mn(VII) (3% of the patch-tested population exhibited irritant reactions to these oxidation states of Mn).

While we patch tested with 2.5% Mn in petrolatum, Mn(II) (or MnCl₂) provided the same number of atoms in suspension as did Mn(VII) (or KMnO₄), whereas Mn(III) (or Mn₂O₃) presented twice the number of atoms in suspension. Despite these differences in potentially bioavailable Mn atoms in suspension during patch testing, irritancy segregated with oxidation state, with a clustering of Mn(III) and Mn(VII) inducing uncommon, TNF- α -independent irritation, and Mn(II) inducing a commoner, TNF- α -dependent irritation. This suggests that biochemistry (ie, oxidation state), rather than bioavailable Mn atoms, is responsible for the observed in vivo and in vitro patterns of inflammation. Equimolar concentrations of these 3 oxidation states of Mn, when studied in vitro (Figs. 2 and 3), confirmed the patch testing data.

These irritant reactions are more likely to occur in whites than in African Americans and those with irritant reactions to other metals. It is possible that it is more difficult to observe subtle erythema in darker skin, possibly explaining the higher observed incidence in white skin than in African American skin. The association of irritant reactions to Mn(II) with that of irritant or allergic reactions to other metals (either in the extended metal series or in the North American series) is interesting and warrants further study.

This high rate of irritation induced by Mn(II) can potentially be mistaken for allergic reactions to Mn(II) if morphologic details of the patch test appearance are not carefully assessed. We demonstrated that Mn(II) is toxic to KCs in a dose-dependent manner; at lower doses, it will be less irritating and therefore less likely to obscure potential allergic reactions. Further studies of serial Mn(II) dilutions for patch testing could be performed to identify the ideal concentration that is capable of eliciting an allergic response while not generating high rates of irritation. Until that is determined, reactions to Mn(II) on patch testing should be carefully evaluated to determine if they are irritant or allergic to avoid misdiagnosis of allergic reactions.

Our in vitro studies of Mn(II), Mn(III), and Mn(VII) correlated with our patch testing results. In vitro, Mn(II) was cytotoxic to KC monolayers, whereas Mn(III) and Mn(VII) were not. In vivo, Mn(II) caused a significantly higher rate of irritant patch test reactions (41% of our patch-tested population) compared with Mn(III) and Mn(VII) (3% of our patch-tested population). We also studied TNF- α release, which is an inflammatory cytokine associated with irritant contact dermatitis, as well as other inflammatory skin conditions.¹² We found that high concentrations of Mn(II) induced significantly higher TNF- α release compared with Mn(III) and Mn(VII).

The reason for a higher rate of toxicity from Mn(II) compared with Mn(III) and Mn(VII) is not understood. This disparity could be due to a difference in the biologic activities of these compounds or other unknown factors. In vitro and in vivo studies of manganese have demonstrated its potential for toxicity, beginning with the first case report published in 1837 of manganese inducing neuropsychiatric symptoms in miners who ground manganese ore.¹³ The neurotoxicity experienced by humans exposed to manganese appears to be related to its oxidation state, with Mn(II) accounting for the majority of known toxicity.¹⁴ The source of this increased toxicity has yet to be established, although a number of in vitro studies have attempted to elucidate this phenomenon. A study of neurons of different types and in different growth stages proved that both Mn(II) and Mn(III) are toxic at high doses, but that the toxicity varies depending on the cell type and the growth phase during which the cells are exposed to manganese. Mn(II) and Mn(III) showed similar toxicities to cerebellar granule neurons; however, Mn(III) was more toxic than Mn(II) toward cultured cortical neurons and PC12 cells. The authors speculate that these different toxicities could be due to different mechanisms of toxicity or levels of accumulation, but could not make any definitive conclusions.¹⁵

There have been few previous studies of the role of oxidation states on allergic or irritant properties. A study of allergy and toxicity induced by chromium (Cr) revealed that Cr(VI) was more allergenic than Cr(III), and 100 to 1000 times more toxic to fibroblasts.¹⁶ The reason for the difference in allergenicity and toxicity of the different oxidation states of Cr is also not well understood. Differing rates of absorption of different oxidation states of manganese may be responsible for the clinical manifestation of higher irritant reactions to Mn(II) compared with Mn(III) and Mn(VII). However, Mn(II) is also more toxic to primary KCs and HaCaT cell line in vitro, a model system where percutaneous penetration is not a limiting factor. These data suggest that Mn(II) is more directly toxic to epidermal KCs when compared with equimolar concentrations of Mn(III) and Mn(VII).

The high rate of irritant reactions to Mn(II) in its current formulation and strength remains an important consideration when patch testing due to its potential to mask allergic responses. This study adds to the data demonstrating that the oxidation state of a metal can influence its ability to cause an irritant reaction. None of the patients tested in our study experienced an allergic reaction to any formulation of Mn, further supporting the notion that allergy to manganese is rare. Given the high rate of irritant reactions, further studies to determine an optimal concentration of Mn(II) to be used in patch testing should be performed.

In conclusion, we describe that MnCl₂ (Mn(II) oxidation state) 2.5% in petrolatum has an unacceptably high rate of irritant patch test reactions in a population of 58 patients undergoing patch testing to evaluate for allergic contact dermatitis suspected to be related to contact allergens in general or related to metal exposures. In vitro studies of MnCl₂ (Mn(II)) with cultured human KCs indicated that this oxidation state, but not Mn(III) or Mn(VII), caused cytotoxicity and release of TNF- α . This suggests that oxidation state of Mn has significant effects on its irritancy and accounts for the enhanced rate of irritant patch test results observed in our patch-tested population.

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Figure 1.

A, Frequency of irritant and allergic reactions at the 48-hour reading of patch tests with the 3 oxidation states [Mn(II), (III), or (VII)] of manganese tested. The majority of patients ($n = 32$ [55%]) had no reaction to any manganese preparations. Mn(II) demonstrated the highest rate of irritant reactions ($n = 24$ [41%]). None of the patch test reactions exhibited allergic morphology to any manganese compound at any of the patch test readings (48, 72, or 96 hours). B, Irritant morphology of Mn (II) patch test reactions at the 48-hour reading from 3 different patients from this study. The irritant reactions noted at the 48-hour readings subsided by the delayed reading, consistent with irritant reactions.

Figure 2.

A, Human foreskin-derived KC survival when incubated with 3 different oxidation states of Mn for 24 hours. There was a significant decrease in KC viability when incubated with Mn(II) compared with the other manganese oxidation states [Mn(III) or (VII)]. This decrease in cell survival appears to be dose dependent. B, Human HaCaT KC cell line was also incubated with 3 different oxidation states of Mn for 24 hours. Only Mn(II) caused a significant decrease in cell survival compared with Mn(III) or Mn(VII) (** $P < 0.001$, * $P < 0.01$, ANOVA).

Figure 3.

Tumor necrosis factor α release into cell-free culture supernatants measured 24 hours after human FSKC or HaCaT cell line monolayers were exposed to 3 different oxidation states of manganese [Mn(II), (III), or (VII)], all at a concentration of 1.544 mM. Mn(II) was capable of inducing significantly greater release of TNF- α compared with Mn(III) or Mn(VII) (** $P < 0.001$, * $P < 0.01$, ANOVA). Dotted horizontal line represents limit of detection of TNF- α enzyme-linked immunosorbent assay (3.2 pg/mL).

TABLE 1**Characteristics of Patch Test Study Population**

n = 58
Sex
Female, 39 (67%)
Male, 19 (33%)
Age, y
Mean, 49
Median, 51.5
Range, 13–83
History of atopy
Positive, 28 (48%)
Negative, 30 (52%)
Race
White, 36 (62%)
African American, 20 (34%)
Hispanic, 1 (2%)
Asian, 1 (2%)

TABLE 2

Bivariate Analysis of Irritant Reactions to Mn

	<u>Irritant Reaction to Mn(II)</u>	<u>Irritant Reaction to Mn(III) and Mn(VII)</u>	<u>Patients With No Reaction</u>
	n = 24	n=2	n = 32
Rate of irritant reactions in male study patients	47.39%	5.26%	47.39%
Rate of irritant reactions in female study patients	38.46%	0%	58.97%
History of atopy	9 (37.5%)	1 (50%)	17 (53%)
Mean age	53	58	46
Smoker	1 (4%)	0	6 (19%)
Allergic or irritant reactions to metals other than manganese	14 (58%, $P = 0.007$)	2 (100%)	6 (19%)
Race	African American: 4 (17%); white: 19 (79%, $P = 0.03$); Asian 1 (4%)	African American: 1 (50%); white 1 (50%)	African American: 15 (47%); white 16 (50%); Hispanic 1 (3%)
Patch tested for suspected metal allergy	7 (29.17%)	1 (50%)	5 (15.63%)