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NICKEL CONTAMINATION OF GOLD SALTS: LINK WITH GOLD-INDUCED SKIN RASH

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SUMMARY

Intramuscular chrysotherapy is a well-established treatment for rheumatoid arthritis. Its therapeutic use has been limited by the high incidence of dermatological side-effects. The pathogenic mechanisms of these are unknown, but could include allergic reactions to gold or to nickel contaminating the gold. In order to investigate these mechanisms further, 15 patients, who developed cutaneous eruptions after chrysotherapy, were assessed using skin biopsy and lymphocyte transformation stimulated by gold and nickel salts *in vitro*. Chrysotherapy induced two main cutaneous eruptions: lichenoid reactions and non-specific dermatitis. Peripheral blood mononuclear cells from patients with lichenoid reaction proliferated to gold salts *in vitro*, while those who developed non-specific dermatitis responded mainly to nickel. Nickel was a significant contaminant of the gold preparation (sodium aurothiomalate, Myocrisin®, Rhone-Poulenc Ltd), amounting to a total of 650 ng after 6 months treatment. We suggest that a significant percentage of skin reactions during chrysotherapy are due to nickel contamination of the gold preparation.

KEY WORDS: Nickel, Gold, Aurothiomalate, Dermatitis, Lichenoid reaction.

GOLD salts, such as disodium aurothiomalate (ATM), containing gold in the gold(I) oxidation state, have been used in the treatment of rheumatoid arthritis (RA) for over 70 yr. They suppress inflammation and retard radiological progression of joint damage [1], but their use is restricted by a high incidence of toxic side-effects. These side-effects include cutaneous eruptions, proteinuria, mouth ulcers, thrombocytopenia and aplastic anaemia. An increased risk of adverse reaction to gold therapy is associated with HLA-B35, DR2 or DR3 [2], as well as the non-HLA-linked slow sulphoxidation status [3]. The commonest side-effect of chrysotherapy is skin toxicity, accounting for up to 60% of all adverse reactions [4]. Penneys *et al.* [5] showed that the most frequent gold-induced cutaneous eruptions were either 'non-specific' dermatitis (NSD) or a lichenoid eruption. Lichen planus is considered to be an antigen-driven T-cell-mediated immune disease since the 'band-like' leucocyte infiltrate is comprised of CD4-positive T lymphocytes. Lichenoid drug eruptions occur commonly after exposure to various chemicals and drugs. Gold(I) is probably the commonest drug causing a lichenoid drug eruption [6].

The exact pathogenic mechanism of ATM-induced lichenoid eruption remains unknown. We have previously shown that gold(III) salts, but not gold(I) salts, are capable of inducing proliferation of lymphocytes in 73% of patients with gold(I)-induced cutaneous eruptions [7]. Gold(III) may be generated by oxidation of gold(I) in the phagolysosomes of activated macrophages and granulocytes, possibly involving myeloperoxidase [8]. The respective roles of gold(I) and gold(III) in inducing adverse immune reactions in mice have been investigated by Schumann *et al.* [9]. They proposed that the immunologically mediated reactions to gold(I) drugs were due to T-cell reactions to gold(III) following *in vivo* oxidation of gold(I).

Recently, Wijnands *et al.* [10] showed that the treatment of one patient with RA, who was known to suffer previously from nickel contact dermatitis with aurothioglucose (Auromyose®), led to the development of an exacerbation of contact hypersensitivity to nickel. They suggested that either the impairment of delayed-type cutaneous hypersensitivity often seen in RA may be reversed by chrysotherapy or that nickel contamination of the gold(I) injections caused the flare in nickel hypersensitivity. The amount of contaminating nickel in the aurothioglucose was 167 ng of nickel per gram of aurothioglucose [10].

There are several case reports of delayed hypersensitivity cutaneous reactions to nickel after the use of infusion cannulas. These cannulas can release nickel in the order of micrograms per litre which is sufficient to cause flares of nickel dermatitis [11–13]. Anaphylactic reactions to nickel have also been described in dialysed patients receiving nickel-contaminated dialysis fluid [14]. The nickel concentration in the blood was only 0.34 ± 0.28 μg/ml compared with 0.28 ± 0.24 μg/ml in healthy volunteers

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[15]. Therefore, a few micrograms of nickel in excess may be enough to provoke flares of dermatitis [16].

Transformation of peripheral blood lymphocytes after their exposure *in vitro* to an antigen to which the donor has become sensitized has been used as a test for drug hypersensitivity [17]. The number of patients who show a positive lymphocyte response to gold(I) drugs is surprisingly small considering the great number of cases with adverse reactions to these drugs [18]. It may be that gold(III) salts are more appropriate recall antigens (see above). Lymphocyte transformation tests can also be used to distinguish between nickel-sensitive and nickel-non-sensitive subjects [19], with 92% of nickel-sensitive subjects having a positive lymphocyte proliferation to nickel sulphate.

To investigate whether nickel contained in gold(I) salts (ATM, Myocrisin®, Rhone-Poulenc Ltd, Dagenham) could be the cause of chrysotherapy-induced cutaneous eruptions, we studied the relationship between blast transformation to gold(III) and nickel(II) salts and cutaneous histology.

MATERIALS AND METHODS

Patients

RA patients treated with ATM (Myocrisin®, Rhone-Poulenc Ltd) who developed a skin rash were recruited from the rheumatology out-patient clinic of Guy's, Greenwich and Lewisham Hospitals. All the patients fulfilled the 1987 American College of Rheumatology criteria for the diagnosis of RA [20]. Rheumatoid factor was positive in 12 out of 15 patients and all but one patient had erosive disease. All the patients were receiving gold treatment when they developed skin rashes. The skin lesions were assessed clinically by a dermatologist (MMB) and the diagnosis was confirmed histologically. In the patients who consented, cutaneous patch tests for gold and nickel hypersensitivity were carried out.

Peripheral mononuclear cell preparation

Mononuclear cells (PBMNC) were isolated from heparinized peripheral blood, taken at the time of discontinuation of chrysotherapy, by Ficoll-Hypaque density gradient centrifugation as previously described [7]. Briefly, peripheral blood was diluted with an equal volume of Hank's balanced salt solution (HBSS; Gibco, Paisley) before being layered onto Lymphoprep (Nycomed Pharma, Oslo, Norway) and centrifuged. Cells collected from the interface were washed three times in HBSS and resuspended at 2×10^5/ml in tissue culture medium (TCM-NHS). TCM-NHS was prepared by supplementing RPMI 1640 with 4 mM L-glutamine, 1% sodium pyruvate, 1% non-essential amino acids, 10 U/ml penicillin/streptomycin (all Gibco, Paisley) and 10% pooled normal human serum (Advanced Protein Products, West Midlands).

Antigen and mitogens

$HAuCl_4$ (Sigma Chemical Co., St Louis, MO, USA) was used as a source of gold(III) and ATM (Myocrisin®, $C_4H_3AuNa_2O_4S$, gold content 49%) for gold(I). The gold compounds were added in concentrations ranging from 1 to 30 μg/ml, $NiCl_2$ (Sigma) from 0.01 to 1 μg/ml and thiomalic acid (Sigma) from 0.5 to 15 μg/ml. Nickel salts were prepared as previously described [19]. Pokeweed mitogen (PWM; Sigma, 0.5 μg/ml) was used as a positive control. All dilutions were in TCM-NHS.

Lymphocyte proliferation

PBMNC triplicate cultures were set up in 96 well U-bottomed plates (Costar Corporation, Cambridge, MA, USA) with or without antigen and PWM in a final volume of 200 μl. Cultures were incubated at 37°C in a 5% CO_2 atmosphere for 6 days. A 0.2 μCi [^{3}H]thymidine pulse (methyl-^{3}H-thymidine, aqueous solution, 5.0 Ci/mmol specific activity; Amersham, Buckinghamshire) was added to the culture for the last 18 h. The cells were harvested and processed for the determination of [^{3}H]thymidine incorporation as disintegrations per minute (d.p.m.). The stimulation index (SI) was calculated as the [^{3}H]thymidine incorporation in the stimulated cultures divided by the [^{3}H]thymidine incorporation of unstimulated cultures. A SI > 2.0 was considered as indicative of a response, with a lower limit of responsiveness of 1000 d.p.m.

HLA typing

Peripheral blood from patients was collected in sodium EDTA. Genomic DNA was isolated using the salt extraction method [21]. The DNA was Class II typed for DRB1/3/4 and 5 alleles, using sequence-specific primers provided in the British Society for Histocompatibility and Immunogenetics (BSHI) kit. The primers, amplification protocol and visualization of the amplified products have been fully described by Olerup and Zetterquist [22, 23].

Nickel measurement

The nickel determination in Myocrisin® was carried out by graphite furnace atomic absorption spectroscopy on a Perkin Elmer 1100B atomic absorption spectrophotometer equipped with a graphite furnace HGA 700. A 10% solution of Myocrisin® (50 mg in 0.5 ml) was mixed 1:1 with aqua regia and heated for 3 h at 100°C to destroy organic matter. It was then further diluted with ultrapure deionized water to a final dilution of 1:10. The concentration of nickel in the sample was then determined by measuring the atomic absorption at 232.0 nm in a pyrocoated graphite tube. The sample was pre-treated at 1000°C and atomized at 2300°C.

Skin biopsies

The skin lesions were assessed clinically and the diagnosis was confirmed by skin biopsies of involved skin. The specimen was divided into two halves and one half was processed for histology and stained with haematoxylin and eosin; the other portion was snap frozen at −70°C and direct immunofluorescence was

then performed. In the patients who consented, cutaneous patch tests for gold(I) and nickel hypersensitivity were carried out using 0.5%, 1% gold(I) sodium thiosulphate, 0.01% gold(I) potassium cyanide and 5% nickel sulphate.

Statistical analysis

Results are expressed as median and interquartile ranges. The difference in SI to gold and nickel was compared using the Mann–Whitney *U*-test. The difference in the number of patients responding positively to gold and nickel, and their relationship to the skin lesion, was compared by χ^2 test.

RESULTS

Fifteen patients were recruited, eight female and seven male, their mean age was 56.3 ± 14.6 yr. The duration of disease was 5.9 ± 6.5 yr and the median cumulative dose of Myocrisin® was 1.7 g (interquartile range 0.78–2.77 g) at the time of discontinuation of therapy. Four of 15 patients (all female) had a lichenoid eruption consisting of discrete erythematous papules and small plaques distributed mainly over the trunk. Some of the lesions were pruritic. One of these four patients had small mouth ulcers. Histology of the skin biopsies confirmed a lichenoid eruption with focal liquefaction and degeneration of the basal cells, and a predominantly lymphocytic infiltrate in the upper dermis. Direct immunofluorescence demonstrated numerous cytoid bodies in the epidermis and papillary dermis consistent with a lichenoid reaction. The other 11 patients (four female and seven male) had an eczematous eruption mainly on the trunk and limbs which was erythematous, scaly and itchy. Histology of the skin biopsies from these patients confirmed an eczematous process with focal spongiosis and an upper dermal perivascular chronic inflammatory infiltrate. Direct immunofluorescence was negative in all these patients. There was no significant difference in the cumulative dosage of gold and duration of RA between the two groups.

Proliferative responses to metals

PBMNC proliferative responses to gold(III) and nickel(II) are shown in Fig. 1. Four patients who developed lichenoid reaction were available for testing; they all showed a significant proliferative response to gold(III) (median SI = 3.6, range 2.5–4.8), but not to nickel(II) (median SI = 0.85, range 0.8–1.65). Only one responded weakly to nickel(II) (SI = 2.4). The difference in SI to gold(III) and nickel(II) was statistically significant ($P < 0.05$). Of the 11 NSD patients, one responded to both gold(III) and nickel(II), three responded to gold(III) alone and four responded to nickel(II) alone. The median SI to gold(III) and nickel(II) were 2.9 (interquartile range 1.6–4.5) and 2.7 (interquartile range 2.4–2.8), respectively. Four patients with NSD showed no proliferation to gold(III) (median SI = 1.1, interquartile range 0.9–1.2) or nickel(II) (median SI = 1.1, interquartile range 1–1.3). None of the patients from either group responded to thiomalic acid (data not shown).

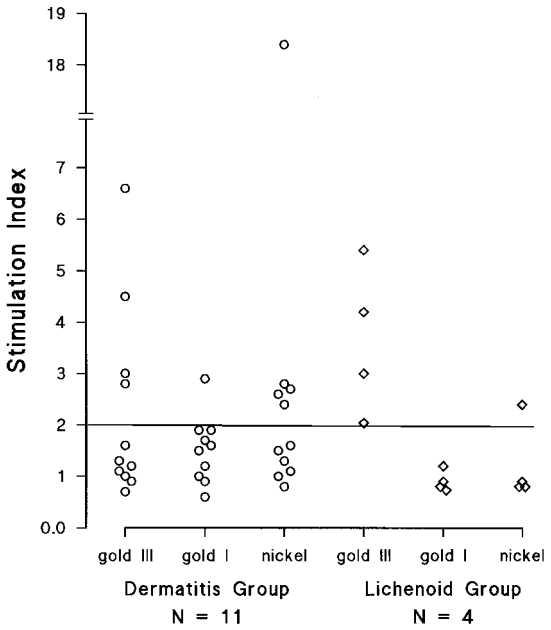

FIG. 1.—Stimulation index to gold(III), gold(I) and nickel(II) in patients who developed dermatitis and lichenoid reactions. A stimulation index >2 was considered to be a positive response.

Nickel measurements

The nickel concentration in native injection solutions of Myocrisin® (50 mg in 0.5 ml) was found to be 0.05 ± 0.008 μg/ml (mean of three measurements).

Patch tests

Patch tests were performed in four patients only. They were all negative to nickel and to gold salts.

HLA types

Tissue typing was performed in 13 patients. In patients with lichenoid reaction, all but one were HLA-DR4 positive and only one patient was HLA-DR3 positive. Five out of 11 patients in the non-specific dermatitis group were HLA-DR4 positive, while two were HLA-DR1 positive. No patient in the non-specific dermatitis group was HLA-DR3 positive. In HLA-DR4-positive patients in the dermatitis group, one responded to gold(III) (SI = 2.8) and one to nickel(II) (SI = 2.4). The others did not show any proliferative response to either gold(III) or nickel(II).

DISCUSSION

The development of skin rash is the principal reason for the early discontinuation of gold salts during treatment of RA. Unfortunately, the onset of the rash often coincides in many patients with the onset of remission of disease activity [24]. Such post-toxicity remission is not seen in patients who experience renal or haematological side-effects [2]. In a previous study, we showed that T-cell proliferation to gold(III) was only observed in patients with gold-

induced dermatitis and not with other forms of toxicity [2].

In this study of 15 cases, we have shown that only four of 15 patients (27%) had a well-defined lichenoid histology. These patients showed peripheral blood T-cell proliferation to gold(III) but not to gold(I) or other metals. It is proposed that in this group of patients the reactive gold(III) moiety induces changes in some component of the immune response—such as a skin-specific constituent, T-cell receptor or major histocompatibility complex molecule—which lead to activation of autoreactive T cells and the accumulation of the $CD4^+$ T cells in the dermis recognized as a lichenoid histological reaction. It may be postulated that this alteration in the patient's immune response is also linked to the improvement in disease activity; a hypothesis which is presently under investigation. When patients were given i.m. depot methylprednisolone acetate over the first 3 months of ATM treatment, there was a suppression of gold-induced dermatitis [25]. This finding supports the notion that ATM-induced skin reaction has an immunological basis.

What is the mechanism of the dermatitis in those patients in whom lichenoid reaction was absent? No correlation was found between the type of skin lesions and the age, sex, duration of RA or cumulative dose of ATM. This raises the possibility that the pathogenesis of ATM-induced cutaneous eruptions may differ. One group showed no T-cell proliferation to either gold(I) or gold(III) or to nickel(II), and the cause of their dermatitis is unclear. The other subgroup of patients with dermatitis showed T-cell proliferation to gold(III) and to nickel(II). One possible explanation for this finding is that ATM is contaminated with nickel and that the repeated injection of ATM leads to nickel sensitization. By contrast, in the patients with a lichenoid eruption, sensitization is specifically to gold. That the double responses in the dermatitis group are indeed only responding to nickel can only be determined by cloning T cells and determining their metal specificity.

In this study, we have shown that ATM used in the treatment of RA was contaminated with traces of nickel. This is not entirely unexpected since nickel-containing alloys are commonly used in the manufacture of industrial apparatus. We have found that ATM contains 0.05 μg/ml nickel which would amount to 650 ng of nickel by 6 months, during which time most cases of dermatitis have already occurred. Thus, in this group of patients, nickel, by unknown mechanisms, causes a dermatitis which may be associated with improvement in disease activity. Our findings on nickel contamination of gold(I) salts are supported by the case report of Wijnands *et al.* [10] showing that aurothioglucose, which is used in some continental European countries, is also contaminated by nickel.

In conclusion, we postulate that nickel-free gold preparations may have reduced toxicity which might make gold a safer treatment in RA.

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