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Assessment of metal sensitizer potency with the reconstructed human epidermis IL-18 assay

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ABSTRACT

According to the new EU Medical Devices (MDR) legislation coming into effect in 2017, manufactures will have to comply with higher standards of quality and safety for medical devices in order to meet common safety concerns regarding such products. Metal alloys are extensively used in dentistry and medicine (e.g. orthopedic surgery and cardiology) even though clinical experience suggests that many metals are sensitizers.

The aim of this study was to further test the applicability domain of the in vitro reconstructed human epidermis (RhE) IL-18 assay developed to identify contact allergens and in doing so: i) determine whether different metal salts, representing leachables from metal alloys used in medical devices, could be correctly labelled and classified; and ii) assess the ability of different salts for the same metal to penetrate the skin stratum corneum. Twenty eight chemicals including 15 metal salts were topically exposed to RhE. Nickel, chrome, gold, palladium were each tested in two different salt forms, and titanium in 4 different salt forms. Metal salts were labelled (YES/NO) as sensitizer if a threshold of more than 5 fold IL18 release was reached. The in vitro estimation of expected sensitization induction level (potency) was assessed by interpolating in vitro EC50 and IL-18 SI2 with LLNA EC3 and human NOEL values from standard reference curves generated using DNCB (extreme) and benzocaine (weak). Metal salts, in contrast to other chemical sensitizers and with the exception of potassium dichromate (VI) and cobalt (II) chloride, were not identified as contact allergens since they only induced a small or no increase in IL-18 production. This finding was not related to a lack of stratum corneum skin penetration since EC50 values (decrease in metabolic activity; MTT assay) were obtained after topical RhE exposure to 8 of the 15 metal salts. For nickel, gold and palladium salts, differences in EC50 values between two salts for the same metal could not be attributed to differences in molarity or valency. For chrome salts the difference in EC50 values may be explained by different valencies (VI vs. III), but not by molarity. In general, metal salts were classified as weaker sensitizers than was indicated from in vivo LLNA EC3 and NOEL data. Our in vitro results show that metals are problematic chemicals to test, in line with the limited number of standardized human and animal studies, which are not currently considered adequate to predict systemic hypersensitivity or autoimmunity, and despite clinical experience, which clearly shows that many metals are indeed a risk to human health.

1. Introduction

Metals have been extensively used in medical devices for many years, in particular in dentistry and orthopedic surgery. Furthermore, metals are generously incorporated into jewelry and many consumer products e.g. sunscreens, food, paint. According to the new EU Medical Devices (MDR) legislation coming into effect in 2017, manufactures will have to comply with higher standards of quality and safety for medical devices in order to meet common safety concerns regarding such products. The current recommendations for testing of almost all

Abbreviations: EC50, chemical concentration resulting in 50% decrease in cell viability; IL-18, Interleukin 18; SI2, 2 fold stimulation index; RhE, reconstructed human epidermis; VUmc, VU university medical center

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medical devices for cytotoxicity, irritation and sensitization are described in ISO 10993-1 which describes how to set up a testing strategy for the safety evaluation of medical devices. In particular, ISO 10993-10 describes tests for irritation and sensitization. With regards to testing sensitization potential of metal salts derived from medical devices, data from animal or human studies performed under standardized conditions is limited. Metals are problematic chemicals to test in the mouse local lymph node assay (LLNA) and in human studies using eg: DSA_{05}, NOEL, LOEL (see ICCVAM database (Basketter et al., 2014; Gerberick et al., 2005; Iccvam, 2011a,b) as ionization followed by binding to a hapten is a primary condition for activation of the human immune system. The correct labeling (sensitizer or not) and classification (potency) of a chemical is important in order to determine the maximum safe concentration for human exposure and to decide whether a less potent sensitizer can replace a more potent sensitizer without effecting the function of the metal alloy.

Despite the lack of robust data from animal and human studies, clinical experience does indicate that a number of metals may be contact sensitizers and may elicit a type IV delayed hypersensitivity reaction in the form of allergic contact dermatitis. Even though metal allergy prevalence in large cohorts is generally unknown (Schedle et al., 2007), there are estimates that up to 17% of women and 3% of men have allergies to nickel and that 1–3% are allergic to cobalt and chromium (Thyssen and Menne, 2010). Mercury, gold and palladium are typical metals used in dentistry that have also been indicated as contact sensitizers with palladium cross reacting with nickel (Gawkrodger, 2005; Muris et al., 2012). Furthermore, rare adverse reactions to titanium containing implants suggests that titanium may also be a sensitizing metal (Wood and Warshaw, 2015; Fage et al., 2016). Testing of potential sensitizers and clinical diagnostic testing for suspected contact allergy is traditionally carried out by applying the metal test chemical in the form of a salt to the skin of an animal of human under standardized conditions. Preferably the salt should dissolve to form metal ions. Metal salts representative of leachables detected in blood and urine are used to apply the ionized metal to the skin of the mouse or human. However, it is generally not taken into account that a number of different salts exist for each metal with different solubility, stratum corneum penetration and cytotoxic/irritant properties which may seriously confound the interpretation of the test results by giving false negative outcomes and under-estimations

Over the last few years, considerable energy has been invested in developing human in vitro methods to identify contact sensitizers. A number of these alternative test methods, e.g. DPRA (OECD-TG 442C), KeratinoSens™ (OECD-TG 442D) and h-CLAT, when incorporated into an Integrated Testing Strategy, are now able to replace animal models such as LLNA for hazard identification (Rovida et al., 2015; Strickland et al., 2016). During the Sens-it-iv Framework 6 project, we developed a reconstructed human epidermis (RhE) in vitro assay for not just identifying contact sensitizers but also for assessing sensitizer potency (Gibbs et al., 2013). RhE consist of proliferating and differentiating keratinocytes grown at the air-liquid interface. Since RhE are cultured exposed to the air, complete epidermal differentiation takes place with the formation of a stratum corneum thus enabling topical chemical application to take place in a similar manner to animal or human testing under standardized conditions. Keratinocytes play a key role in sensitization and activation of the immune responses as described in the Adverse Outcome Pathway for sensitization (Rovida et al., 2015). The differentiated epidermis controls chemical bioavailability via the stratum corneum and the underlying viable keratinocytes trigger, via the inflammasome and NF-kB pathway, an inflammatory response in the form of (pro-) inflammatory cytokine release (Martin, 2015a). Among the many cytokines secreted by keratinocytes, IL-18 has been shown to play a key role in induction of allergic contact dermatitis (sensitization) by influencing the migration of Langerhans cells and dendritic cells to the draining lymph nodes, and in turn the presentation of the haptenized proteins to T cells (Antonopoulos et al., 2008; Okamura et al., 1995). IL-18 has no apparent role in irritant contact dermatitis, indicating that the role of IL-18 in contact hypersensitivity is not simply part of a general requirement for IL-18 in skin inflammation (Antonopoulos et al., 2008). IL-18 plays a pivotal role in sensitization since it promotes a Th1- type immune response by enhancing the secretion of pro-inflammatory mediators such as TNFα, CXCL8 and IFNγ (Okamura et al., 1995; Cumberbatch et al., 2001). Importantly, we have shown that IL-18 can now be used to identify contact sensitizers from respiratory sensitizers and non-sensitizers in an RhE in vitro assay (Gibbs et al., 2013; Andres et al., 2017; Galbiati et al., 2017). The RhE IL-18 assay could identify with 95% accuracy a panel of 17 contact sensitizers. Potency assessment correlated better with human DSA_{05} data which assesses the induction dose per skin area that produces a positive response in 5% of the tested population than with LLNA data (Gibbs et al., 2013). The assay was extremely transferable from the in house VUmc model to commercially available RhE (Gibbs et al., 2013; Andres et al., 2017; Teunis et al., 2014). In the assay prediction model (Gibbs et al., 2013), depending on the RhE used, a chemical is labelled (YES/NO) as a sensitizer if, in 2 out of 3 independent runs, a threshold of $\geq$ 5 fold IL-18 release into the culture supernatant occurs in order to avoid irritants scoring as false positive. Potency, on the other hand which is related to the irritant potential of the chemical, is assessed by the chemical concentration resulting in 50% decrease in cell viability (EC50) or in 2 fold increase in IL-18 release (SI2). By definition therefore, potency is related to the irritant potential of the chemical and does not distinguish a sensitizer from a non-sensitizer: the stronger the sensitizer the lower the EC50 or IL-18 SI2 value will be (Dos Santos et al., 2011; Spiekstra et al., 2009). In line with this, the irritant capacity of chemicals has long been clinically recognized to represent an additional risk factor for sensitization induction (Agner et al., 2002; Basketter et al., 2007; Bonneville et al., 2007; Grabbe et al., 1996; Mclelland et al., 1991).

In this study, we expand on our recently published study that describes the use of the RhE assay to estimate the expected sensitization induction level by interpolating in vitro EC50 and IL-18 SI2 values to predict LLNA EC3 and/or human NOEL from standard curves generated using reference contact sensitizers (Galbiati et al., 2017). In order to test the sensitizing potential of metal salts as replacement for metal ions leaching from routinely used medical devices further, and to gain more insight into the mechanism by which different metal salts for the same metal may influence the read out of the current skin patch test, we tested a panel of 28 chemicals consisting of 17 metal salts, 8 non-metal sensitizers and 4 non-sensitizers (including zinc chloride) in the RhE IL-18 assay. For four metals (nickel, chrome, gold, palladium), the same metal was tested in two different salt forms, and titanium was tested in 4 different salt forms to investigate the influence of molarity, valency and cytotoxicity on the outcome of the assay.

2. Materials and methods

2.1. Reconstructed human epidermis

Healthy human neonatal foreskin was obtained after informed consent from patients undergoing routine surgical procedures. Skin was used anonymously and in accordance with the “Code for Proper Use of Human Tissue” as formulated by the Dutch Federation of Medical Scientific Societies (www.fmwv.nl), and following procedures approved by the VU University medical center institutional review board.

VU University medical center in-house RhE (VUmc-RhE) were used in this study. RhE were constructed from human foreskin keratinocytes as described previously (Dos Santos et al., 2011; Spiekstra et al., 2009). In short, keratinocytes (passage 2) were seeded into a 12 mm diameter transwell (pore size of 0.4 mm; Corning, NY, USA) and grown submerged in medium containing DMEM/Hams F12 (3:1), 1% ultroserG, 1 μM hydrocortisone, 1 μM isoproteronol, 0.1 μM insulin and 1 ng/mL KGF for 1 week. Cultures were then lifted to the air–liquid interface and