FreeMet® technology prevents heavy metal ions uptake in human skin cells.

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Abstract

The worsening of environmental conditions induced by human activity such as mining, metal smelters, burning of fossil fuels, or soil contamination; it has produced an increase in pollutants leading to the exposition of human beings to noxious particles like heavy metals. This has led to increased incidence of pathologies in different tissues, where the skin is one of the most affected as it is directly exposed to these contaminants. In this work, we present a preparation based on a mixture of FDA-approved molecules aiming to block the uptake of heavy metals ions by human skin cells, hence preventing skin-related diseases derived from heavy metal exposure.. Here, we demonstrated that incubation of epidermal skin cells with FreeMet® technology completely prevents heavy metal ions uptake in terms of determining intracellular levels of lead, mercury, cadmium and arsenic. Furthermore, this formulation could be used as a heavy metal scavenger to maintain a healthy skin.

Keywords: keratinocytes, fibroblasts, heavy metals, skin

Introduction

The denomination of heavy metals is assigned to metallic elements that have an atomic weight greater than the atomic mass of calcium (40.08). These elements are naturally present in The Earth crust, but in the last few decades their presence in the environment is increasing as a result of human being activities (Table 1) such as mining, industrial discharge, urban runoff, sewage effluents, application of pesticides to plague control in plants, burning of fossil fuels, metal smelters, etc.[1] Although some of these elements (i.e. iron, cobalt, copper, manganese, molybdenum, and zinc) are indispensable for humans,[2] such as copper, which is a cofactor of some metalloenzymes,[3] they become toxic when the cells are exposed to elevated concentrations of these elements.[4] These new contaminants, as important parts of air pollution, generate a continuous exposure of humans to these elements in high concentrations, when they become toxic for cells. Some elements with high degree of toxicity are arsenic, cadmium, lead and mercury, which are ranked as priority metals of public health relevance.[5] These elements are considered toxic as they induce multiple organ damage, even at low levels of exposure.[5] One of the recognized noxious effect of heavy metals on animal cells is that they are carcinogenic elements by themselves, and in addition they promote increased oxidative stress that damages the plasma membrane, leading eventually to cell death.[6]

Table 1. Sources of heavy metals related to human being activities, their consequences on health and permissible levels

Heavy metals	Main sources from human beings activities	Some human health issues	Permissible levels (mg/l)
Arsenic	Pesticides, metal smelters, mining	Dermatitis, poisoning	0.02[(2)]
Lead	Paints, pesticides, smoking, automobile emission, mining	Gastrointestinal damage, mental retardation in children	0.1[(2)]
Cadmium	Pesticides, welding, Cd and Ni batteries	Cancer, lung disease	0.06[(2)]
Mercury	Pesticides, paper industry	Poisoning, damage to the nervous system	0.01[(2)]

[(2)] Data obtained from reference 2.

The skin is the first organ to come into contact with these elements present as air pollutants. Hence, it is advisable to protect skin cells from the noxious effect of these elements.

Skin tissue is the largest organ in the body. It fully covers the external surface of the body, serving as a first-order barrier against pathogens and chemicals (like pollutants), and provides a mechanical barrier to injury. This tissue is composed of two main layers divided in sublayers; 1) the epidermis, the most superficial layer composed of a keratinized, stratified squamous epithelium, formed by keratinocytes and 2) the dermis, composed of connective tissue, blood vessels, hair follicles, sweat glands, and other structures.[7]

Since, the skin is the first barrier against chemicals or air pollution contaminants, like heavy metals, it is very necessary to avoid the entry of these elements to skin cells in order to protect them and maintain a healthy condition. In this work, we assayed the capacity of FreeMet® technology, which is a formulation of two FDA approved molecules, as heavy metal scavengers and possible skin protector.

Results

Divalent or trivalent heavy metal ions (Cd^{2+}, Hg^{2+}, Pb^{2+} and As^{3+}) are spontaneously taken up by keratinocytes and fibroblasts cells.

As previously mentioned, heavy metals are part of the air contaminants. Thus, skin tissue is frequently exposed to these elements. We therefore evaluated if keratinocytes, as part of the most external skin layer (epidermis), and fibroblasts skin cells, as part of the inner layer (dermis), could spontaneously incorporate divalent or trivalent heavy metals in their cytoplasm as previously reported for HeLa or KB cells.[8,9] A keratinocytes cell line, HaCat, was incubated with probes that detect Cd^{2+}, Hg^{2+}, Pb^{2+} and As^{3+} at a concentration of 50 μM for 60 min. Once loaded with the probes, the cells were incubated in the presence of heavy metals and the uptake of these elements was determined after 45 min of incubation (Fig. 1). The same procedure was followed in primary cultures of skin fibroblasts (Fig. 2), detecting that all heavy metal ions evaluated here were spontaneously taken up by both keratinocytes and fibroblasts epidermal cells (Fig. 1 and 2).

FreeMet® technology prevented the heavy metals uptake in both keratinocytes and fibroblasts skin cells

Keratinocytes and fibroblasts skin cells previously loaded with probes were incubated with FreeMet® technology (1% w/v) 30 min before heavy metals salts were added to the external media, in order to evaluate whether this formulation is capable of inhibiting heavy metal uptake into skin cells. Fig 1 and 2 show that incubation with FreeMet® technology completely blocked the entry of these elements into the cells, suggesting that this compound could constitute a skin protector against the presence of heavy metals.

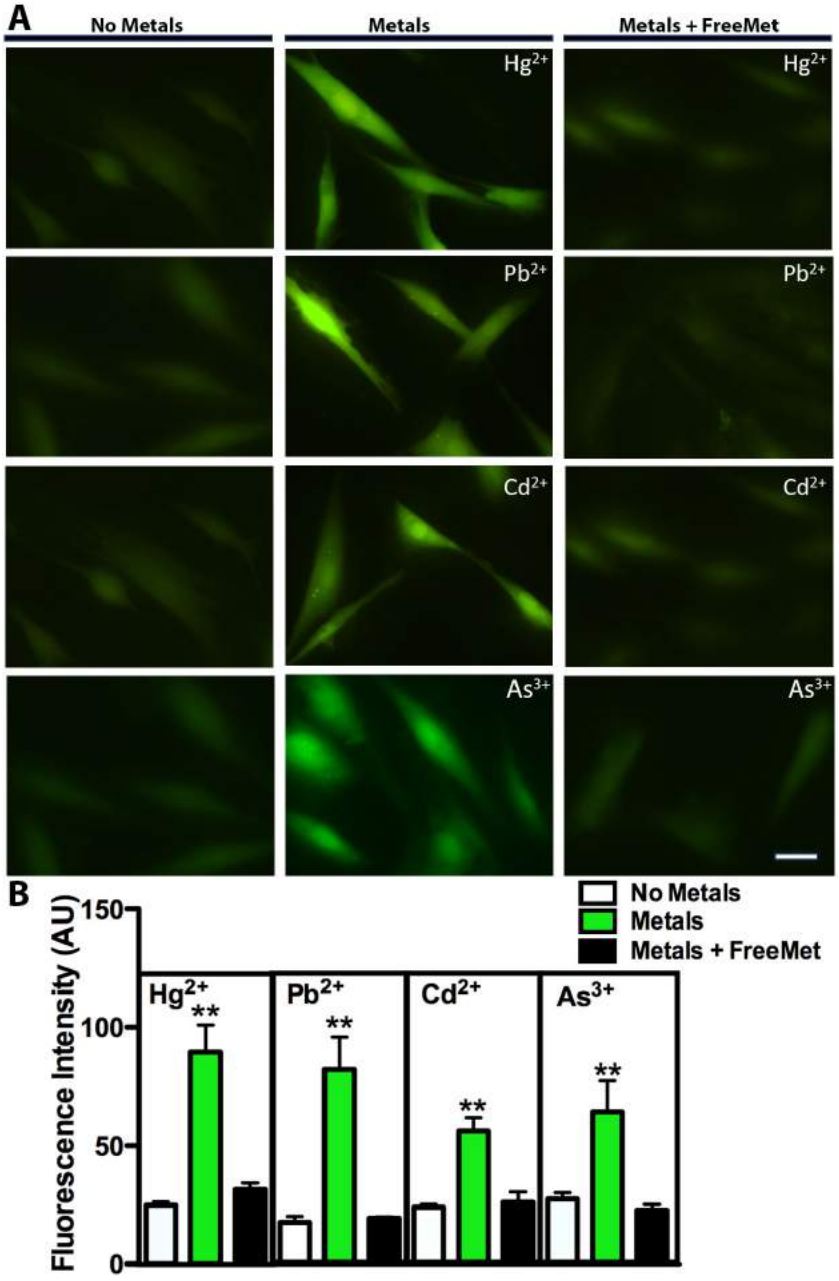

Figure 1. Heavy metal uptake in human skin keratinocytes cells is prevented by the FreeMet formulation. Human skin keratinocytes were loaded with a probe that detects divalent cations or a probe that detects trivalent cations and were later exposed (40 min) to different heavy metals ions like arsenic, mercury, lead and cadmium. These experiments were performed in cells incubated previously with FreeMet. **A.** Detection of heavy metal ions inside the cells (green signal), representative images. **B.** Analysis of the data shown in **A**. Scale bar: 10 μm

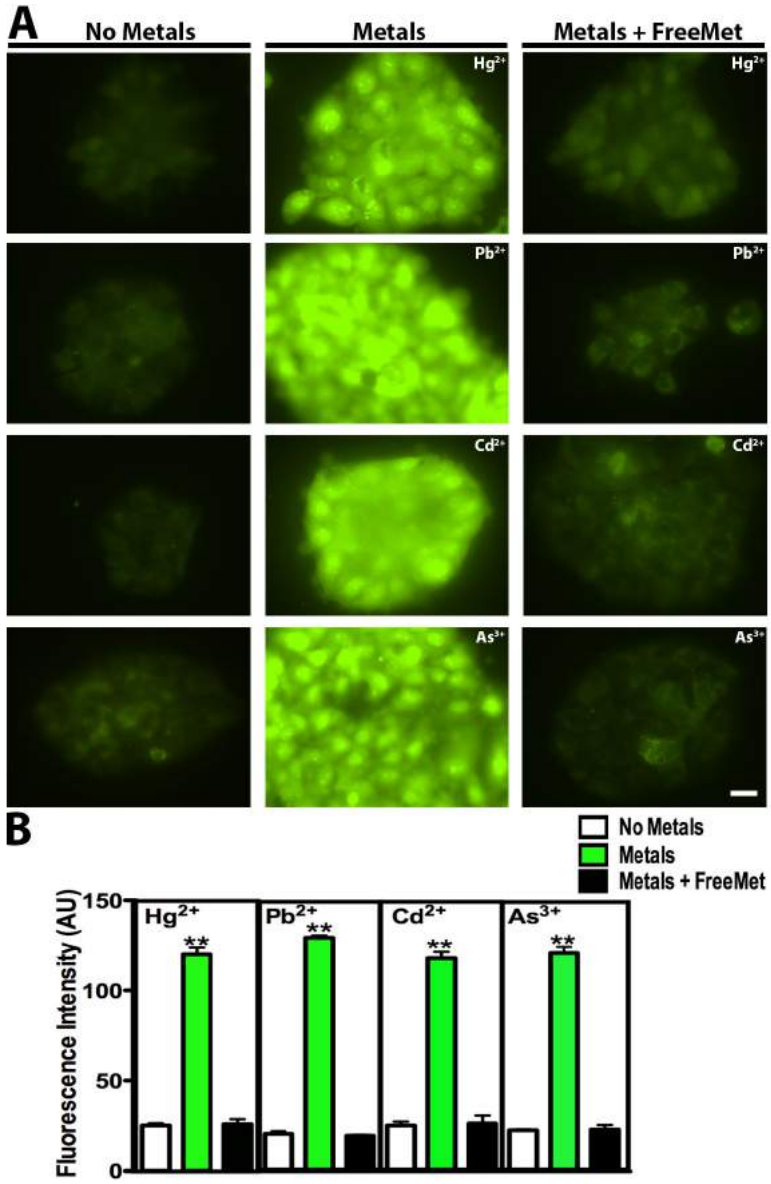

Figure 2. Heavy metal uptake in human skin fibroblast cells is prevented by the FreeMet formulation. Human skin fibroblasts were loaded with a probe that detects divalent cations or a probe that detects trivalent cations, and were later exposed (40 min) to different heavy metals ions like mercury, lead, cadmium and arsenic. These experiments were performed in cells incubated previously incubated with FreeMet. **A.** Detection of heavy metal ions inside the cells (green signal) representative images. **B.** Analysis of the data obtained in **A**. Scale bar: 10 μm

Methods

Reagents. Probes Phen Green FL (for determination of Hg^{2+}, Pb^{2+} and Cd^{2+}) and Newport Green™ (for determination of As^{3+}) were obtained from Thermofisher Scientific (Waltham, MA, USA). Cadmium dichloride, mercury dichloride, lead dichloride and Arsenic trichloride were obtained from Sigma-Aldrich (Chicago, IL, USA).

Skin fibroblasts culture. Primary cultures of human skin fibroblasts were obtained from (Inbiocriotec, Valparaíso, Chile) and the cells were cultured with proliferation media (DMEM supplemented with 10 % fetal bovine serum). Once the cells attained confluence, they were seeded onto glass coverslips to later perform heavy metal ion uptake assays.

Ketaratinocytes culture. A human keratinocyte cell line HaCat was grown in DMEM medium supplemented with 10 % fetal bovine serum. Once confluence was attained, the cells were seeded onto coverslips (12 mm) and used for analysis of heavy metal ions uptake.

Heavy metal ions uptake assay. Human fibroblast or keratinocytes cells were loaded for 30 min with a probe that recognize divalent heavy metal ions (Hg^{2+}, Pb^{2+} and Cd^{2+}) or a probe that detect As^{3+}. Then, a solution containing heavy metal ions was added to the cells for 45 min. The uptake of heavy metal ions was analyzed by fluorescence microscopy, recording the emission at 540 nm (green signal) inside the cells. In the assays performed in the presence of the FreeMet® formulation, cells were incubated with the mixture for 30 min. FreeMet® was then removed, and a solution containing heavy metal ions was added for 45 minutes. Afterwards, the uptake of heavy metals was analyzed as previously described.

Discussion

In this work, it was demonstrated that FreeMet® completely prevented the entry of the heavy metal ions Cd^{+2}, Hg^{+2}, Pb^{+2} and As^{+3} in two types of skin cells keratinocytes derivatives from the epidermis layer, as well as in fibroblasts from the dermis layer, hence potentially protecting them from possible air pollutant toxicity. The skin is the largest organ in the human body, which it covers completely. Thus, the skin may confer protection from different external stimuli like temperature, pathogens, radiation and exposure to chemicals.[10] However, this protection is not assured as any noxious stimuli of a different nature, for example, like UVB radiation[11] that affects normal cell functions, could lead to lose this protection. In addition, it is important to point out that this tissue represents the main means of exposure to heavy metals in adults and children.[12] The chronic exposure to heavy metals induce cell death.[4] Furthermore, skin care is absolutely necessary for the preservation of appropriated tissue function. The effeciency of the FreeMet® formulation in protecting skin cells against air pollutants relies on a broad spectrum of well-recognized, pharmacological and therapeutic properties of each of the single active ingredients, which include antimicrobial and anticancer properties. Finally, these compounds are biodegradable, biocompatible and ecofriendly.[13] The FreeMet® formulation could represent an interesting alternative to maintain skin health in the health and cosmetic industry.

Acknowledgments

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Conflict of interests

CU and AM are employees of Cyra Consultora Ltda, a for-profit company. The authors report no other conflicts of interest in this work.

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