The permeability of magnesium across the skin is enhanced by menthol cream

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Abstract. Magnesium (Mg^{++}) contributes to several normal physiological functions. Some topical Mg^{++} products are marketed for Mg^{++} deficiency, but the rate and extent of topical Mg^{++} absorption are not known. Our aim was to compare the permeation of Mg^{++} across porcine skin from 1 % (m/m) Mg^{++} creams with and without levomenthol. Permeability studies were performed with the Franz cell model, and the Mg^{++} concentrations were quantified with inductively coupled plasma-mass spectrometry. The flux of Mg^{++} across porcine skin was higher with levomenthol-based cream, for a median value of 29.7 µg/cm^2/32 hr, than that from the cream without menthol, which was 6.2 µg/cm^2/32 hr. The median apparent permeability (P_{app}) value was $2.6 \cdot 10^{-8}$ cm/sec (range 0.44 - 8.1) from levomenthol-based cream and $0.9 \cdot 10^{-8}$ cm/sec (range 0.1 - 3.8) from the cream without menthol. This study confirmed that Mg^{++} can permeate through porcine skin and that coadministration with levomenthol substantially enhanced the permeability.

Key words: magnesium, porcine skin, permeability, levomenthol

Introduction

Magnesium (Mg^{++}) is a cofactor in hundreds of enzymatic reactions and is essential for many physiological functions, such as heart rhythm, vascular tone, nerve function, and muscle contraction and relaxation. Mg^{++} is also needed for bone formation, and it regulates calcium, potassium, and sodium homeostasis. The Mg^{++} demand is increased in, for example, pregnant women and athletes [1]. Food is the main source of Mg^{++}, but epidemiological studies indicate that half of the adult population consumes less than an adequate daily intake of 300-350 mg Mg^{++} from food [2].

Magnesium supplements given by mouth are commonly used to treat Mg^{++} deficiency. This route of administration is often well tolerated, but it may cause dose-dependent gastrointestinal symptoms, including nausea, vomiting, and diarrhoea [3]. Currently, there are commercially available Mg^{++}-containing cream products that

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are applied topically on the surface of the skin. Skin, and especially the outermost layer of the skin, the *stratum corneum*, is one of the tightest barriers in the human body. Only very small and lipophilic molecules are able to penetrate across the skin. The permeability of Mg^{++} through the skin has not been established. It is assumed to depend on pathways related to appendages, glands, hair follicles, and the hydration state or integrity of the skin [4].

The permeability of the skin can be increased by the excipients termed permeation enhancers. Menthol is one compound with penetration-enhancing properties [5]. Menthol is a natural fat-soluble terpene that can permeate the epidermis. Menthol enhances transdermal penetration of other substances through the outermost layer of the skin by interacting with intracellular lipids and therefore interfering with the lipid structure [5]. It might also act as an enhancer via calcium ion interactions [6].

In this study, we investigated the extent of Mg^{++} permeation through porcine skin from creams with or without levomenthol by the Franz cell chamber model.

Materials and methods

Product

Two creams, one with and one without levomenthol (but otherwise equal in composition), were kindly provided by Fysioline Oy, Tampere, Finland. The content of levomenthol-containing cream was identical to commercially available Magnesium In-cream (Fysioline Oy). Both creams contained 10 % (m/m) Mg-sulfate ($MgSO_4 \cdot 7\ H_2O$) corresponding to a Mg^{++} concentration of 1 % (m/m).

Porcine skin

Porcine skins were obtained from animal studies performed at the University of Eastern Finland, Kuopio, Finland. The skin samples were excised immediately after sacrification from porcine abdomens. The epidermis was peeled off the skin after heating the sample in distilled water (2 min at +60 °C). The separated epidermis was dried and stored at -20 °C for later use.

Permeability Test

Permeability studies were performed by using a widely used and accepted skin permeation model, the Franz cell chamber method [7]. The skin was hydrated in phosphate-buffered saline (PBS) for 10-15 min and placed into a chamber. The surface area of the skin was 0.64 cm^2. The formulation was placed in the donor compartment (0.5 g, containing 5 mg of Mg^{++}), and samples of 0.5 mL were collected from the receiver side at 1, 2, 4, 6, 8, 12, 24, 28, and 32 hr. The samples were stored at -20 °C until the quantitative analysis of Mg^{++}. Skin permeability experiments were performed in two consecutive experiments. In the first experiment, we had 7 sets of cream with levomenthol and 6 sets of cream without levomenthol, and vice versa in the second experiment. A few chambers were excluded due to leakage in both the groups, and thus, we had data for 9 chambers without levomenthol and 9 chambers with levomenthol cream.

Quantitative Analysis

The magnesium concentration of the samples was measured by inductively coupled plasma-mass spectrometry (ICP-MS) using a NexION 350D ICP-MS (PerkinElmer Inc., Waltham, MA, USA) and an ESI PrepFAST autosampler (Elemental Scientific, Omaha, NE, USA). A triple-quadrupole reaction system was used to remove polyatomic interferences. The reaction system was operated in collision mode with kinetic energy discrimination (KED) using helium as the cell gas (3.7 mL/min). A peristaltic pump and nebulizer were used for sample injection. The internal standard, scandium-45, was mixed online with the samples to compensate for matrix effects and instrumental drift. Mg^{++} was determined against a certified multi-element calibration standard (TraceCERT Periodic Table Mix 1, Sigma Aldrich, Merck KGaA, Darmstadt, Germany) in acidic conditions (2.5 % HNO_3, TraceMetal™ grade, Fisher Chemical, Hampton, NH, USA). The calibration range used for Mg^{++} was 4–400 µg/L, and the detection limit (LOD) was 0.16 µg/L. Before measurements, the samples were diluted within the calibration range using 2.5 % HNO_3. The instrument was operated with an RF power of 1.6 kW and with nebuliser gas, auxiliary gas, and plasma gas

flows of 0.92, 18, and 1.2 L/min, respectively. The sample uptake rate was 3.5 mL/min, and dwell times were set to 50 ms per AMU. Three replicates were obtained for each sample. The data were processed using PerkinElmer Syngistix data analysis software™.

Calculations and statistics

The apparent permeability coefficient (P_{app}) values were calculated as follows:

$$P_{app} = J/(C_o \times A)$$

where J is the drug flux (linear range) across the skin, C_o is the initial concentration on the donor site, and A is the surface area of the skin.

The two groups were compared with the Mann-Whitney U-test. A null hypothesis was rejected if the p-value was less than 0.05.

Results

The results showed that Mg^{++} could permeate through porcine skin to a certain extent. The flux of Mg^{++} across porcine skin ranged between 5.8 and 82.3 μg/cm^2/32 hr (median 29.7) in the menthol-containing group (*figure 1A*) and between 1.8 and 33.0 μg/cm^2/32 hr (median 6.2) in the menthol-free group (*figure 1B*), ($p = 0.031$). In a *post hoc* analysis, it appeared that the Mg^{++} flux was similar in the two groups during the first 12 hours (at 1 hour $p = 0.397$; at 2 hours $p = 0.605$; at 4 hours $p = 0.863$; at 6 hours $p = 0.605$; at 8 hours $p = 0.340$; at 12 hours $p = 0.094$), but thereafter it was significantly higher in the menthol-containing group than in the menthol-free group (at 24 hours $p = 0.031$; at 28 hours $p = 0.019$) (*figure 1A and 1B*).

The apparent permeability (P_{app}) values ranged between 0.44×10^{-8} and 8.1×10^{-8} cm/sec (median 2.6) in the menthol-containing group and between 0.1×10^{-8} and 3.8×10^{-8} cm/sec (median 0.9) ($p = 0.105$) in the menthol-free group, respectively. Figure 1 shows that levomenthol enhanced the skin permeability of Mg^{++}, although the difference between P_{app} values was not statistically significant.

Discussion

Magnesium is an essential mineral and is involved in up to 80 % of metabolic functions in the human body [8]. Based on scientific evidence, the EU has approved eleven health claims of Mg^{++}, such as contributions to normal psychological functions, maintenance of the immune system, and normal muscle contraction [9].

In this study, we have shown that Mg^{++} can penetrate through porcine skin. As the permeability of porcine skin is quite similar to human skin [10], we assume that the data obtained here are also valid in human skin. The obtained P_{app} values of Mg^{++} across porcine skin are very low from the cream without menthol. For example, fentanyl and buprenorphine, which are used for analgesia and are commercially available as transdermal patch products, have 10-100-fold higher P_{app} across the skin [11, 12]. Chandrasekaran et al. [4] showed in their studies based on multiphoton microscopy that the hair follicles and sweat glands account approximately half of the permeation of the Mg^{++} permeation across the human *stratum corneum*. This is a reasonable explanation since the epithelia in skin appendages are known to be leakier than the *stratum corneum*. Mg^{++} is not able to penetrate through the highly lipophilic and densely packed *stratum corneum* due to its highly ionized nature. The surface area of appendages is less than 0.1 % of the total skin surface area, and therefore, this route is considered insignificant in transdermal drug delivery.

The use of permeation enhancers is needed to improve the permeability of Mg^{++} across the skin. We showed that levomenthol increased the permeation of Mg^{++} across porcine skin substantially. This is consistent with earlier data indicating that menthol has penetration enhancing properties [5, 6]. However, the increase in Mg^{++} skin permeability was not evident during the first hours in this experimental model, indicating that in clinical use Mg^{++}-levomenthol cream should be applied on skin on regular basis to obtain any meaningful absorption.

Magnesium permeation from the cream without levomenthol was similar to that reported by Mayuramas et al. [13] in a study on isolated human cadaver skin. They concluded that transdermal Mg^{++} may be used to treat symptoms of Mg^{++} deficiency. However, as the amount of Mg^{++} permeation from the menthol containing cream was 4-5-fold higher from the levomenthol-based cream, we believe that this is more appropriate formulation for clinical use. Howev-

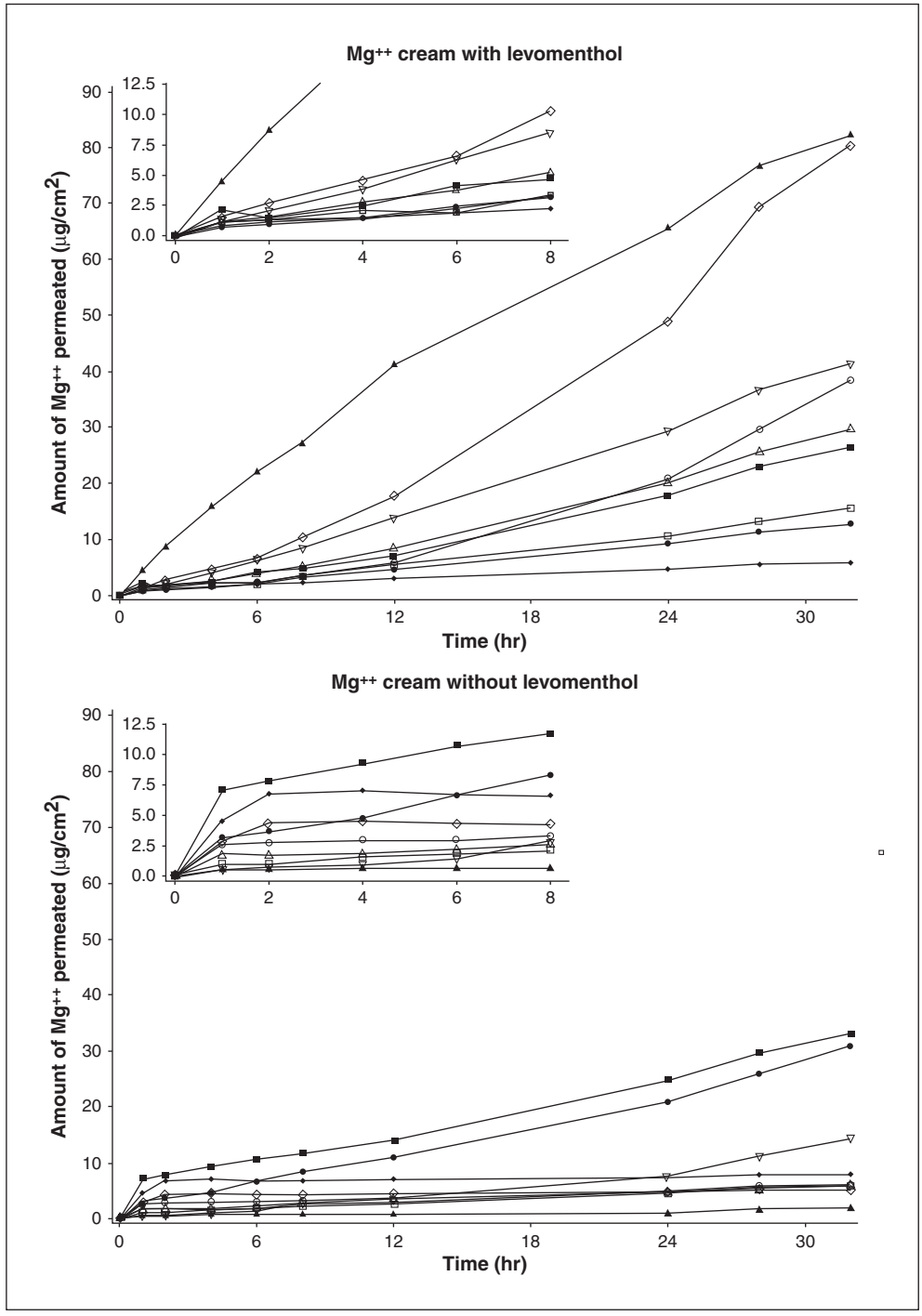

Figure 1. Permeation of Mg^{++} across porcine skin as a function of time from levomenthol-containing cream (A) and cream without levomenthol (B). Each curve represents the drug flux in one chamber. P = 0.031 between the two groups.

er, in case of hypomagnesemia there is a low probability that topical Mg^{++} administration could significantly contribute to Mg^{++} body status [14]. Further data are needed to show whether Mg^{++}-levomenthol cream can be applied topically to the surface of the skin to achieve systemic Mg^{++} concentrations that have therapeutic effects.

There is an interest in topical Mg^{++} administration in dermatology as it reduces inflammation. Topical Mg^{++} is known to bind water, influence epidermal proliferation and differentiation, and enhance permeability barrier repair [15, 16]. Magnesium is used, e.g., in atopic dermatitis. Menthol is also used in skin conditions for centuries as it has antipruritic activity, pruritus is a common complaint in many skin diseases. Menthol is used as an antiseptic, as it has bacteriostatic, antiviral, and antifungal activity [17]. Moreover, as topical menthol increases skin blood flow [18] and has anti-inflammatory activity [19], we assume Mg^{++}-levomenthol combination could be a useful combination in skin diseases also.

There are some limitations in the present study. This was an experimental study. However, human studies would be challenging to accomplish. First, since Mg^{++} is distributed and compartmentalized within the body into different locations (0.3 % in serum, approximately 25 % in muscle and mainly in bones), determining the Mg^{++} content and distribution within the body is a major demand [1, 8]. Total body Mg^{++} depletion can be present with normal plasma Mg^{++} concentrations, and there can be hypomagnesaemia without total body deficiency [20]. Second, Mg^{++} homeostasis within the body is normally regulated in the short term by the kidneys, and in the longer term, it is affected by bone storage also. Urinary Mg^{++} concentrations are poor indicators of Mg^{++} status, as the kidney has a large capacity to compensate for Mg^{++} absorption [8]. Third, the use of certain drugs may cause bias in determining Mg^{++} status: antacids and proton pump inhibitors substantially decrease Mg^{++} absorption from the small intestine, tetracyclines and corticosteroids interact with Mg^{++} to form insoluble crystalline compounds, and diuretics not only bind to Mg^{++} but also increase its renal excretion. Other compounds that can increase Mg^{++} renal excretion include coffee, alcohol, and oral contraceptives [8].

We assume that this Franz cell model is a reliable way to demonstrate in a laboratory setting that levomenthol has an effect on Mg^{++} permeation through the s*tratum corneum*. This Franz's method has been widely used in studies on percutaneous permeation and in the determination of the pharmacokinetics of topically applied compounds [7]. Our data indicate that this method is feasible for evaluating the effect of penetration enhancers such as levomenthol in this study.

Conclusion

The data indicate that Mg^{++} can permeate through porcine skin and that coadministration with levomenthol enhanced the permeability substantially.

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