Arch Dermatol Res (1999) 291 : 560–563

ORIGINAL PAPER

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Some magnesium salts and a mixture of magnesium and calcium salts accelerate skin barrier recovery

Received: 16 March 1999 / Received after revision: 17 June 1999 / Accepted: 18 June 1999

Abstract **The effects of four different magnesium salts on the cutaneous barrier recovery rate after barrier disruption were evaluated. We spread an aqueous solution of each salt on the flank skin of hairless mice, occluded the area with a plastic membrane for 20 min, and then left the skin surface to dry. All of the magnesium salts, except magnesium bis(dihydrogen phosphate), accelerated barrier repair. We next estimated the effects of magnesium chloride aqueous solutions which contained calcium chloride at different molar ratios. When the calcium to magnesium ratio was lower than 1, the mixture accelerated barrier repair. The application of an aqueous solution of 10 m*M* magnesium chloride and 10 m*M* calcium chloride was found to hasten the barrier recovery more effectively than a solution of 10 m*M* magnesium chloride. These results suggest that the effects of these metal ions are different depending on the counter ion and/or the method of application.**

Key words Magnesium salts · Calcium salts · Skin barrier

Introduction

The cutaneous permeability barrier is located in the outermost layer of the skin, the stratum corneum (SC). Acute disruption of the barrier by organic solvents, detergents, or tape stripping elicits a homeostatic response in the epidermis which rapidly results in restoration of the barrier function [1]. There is a Ca^{2+} gradient in the epidermis with low concentrations in the basal layer and progressively higher concentrations towards the upper epidermis [2]. This Ca^{2+} gradient disappears immediately after acute barrier disruption [3]. In the epidermis of chronic dermatitis, such as atopic dermatitis, which is associated with barrier abnormalities, the Ca^{2+} gradient is also abnormal [4]. During the fetal development of skin, the Ca^{2+} gradient forms coincident with the emergence of barrier competence [5]. Destroying the Ca^{2+} gradient by sonophoresis perturbs barrier homeostasis [6]. Thus the calcium ion appears to play a crucial role in skin barrier homeostasis.

Zglinicki et al. have demonstrated that magnesium concentrations are also high in the SC but constantly low in all other strata [7]. However, the role of magnesium in epidermal barrier homeostasis has not been investigated. Lee et al. have reported that the topical application of calcium ions delays cutaneous barrier recovery [8]. They have also demonstrated that potassium, phosphate and magnesium modestly inhibit barrier repair [8]. We report here that the topical application of magnesium ions by different methods gives different results, and also report the paradoxical effect of a mixture of magnesium and calcium salts on the barrier repair processes.

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Material and methods

Animals

Hairless mice (HR-1, Hoshino, Japan) were used at 7–10 weeks of age. Before the experiments, animals were caged separately for at least 4 days. The cages were kept in a room at 21–25 °C and a relative humidity of 40–70%. All treatments were carried out under anesthesia and all experiments were approved by the Animal Research Committee of the Shiseido Research Center in accordance with National Research Council (NRC) guidance [9].

Materials

Magnesium chloride, magnesium sulfate, and magnesium lactate were purchased from Wako, Japan. Magnesium bis(dihydrogen phosphate) was purchased from Junsei Chemical Company, Japan and calcium chloride was purchased from Nakarai Tesque, Japan. Dulbecco's phosphate-buffered saline (PBS) was purchased from Life Technologies (Grand Island, N.Y.).

Barrier disruption and its recovery rate

The flank skin of mice was subjected to tape stripping, as described previously [10]. The procedure was terminated when transepidermal water loss (TEWL) reached 7.0–10.0 mg/cm^2 per h. Normal TEWL is less than 0.2 mg/cm^2 per h. TEWL was measured with an electrolytic water analyzer (MEECO, Warrington, Pa.), as described previously [10]. Immediately after barrier disruption, 50 µl of each aqueous solution was applied and the area was occluded with a plastic membrane (2 × 3 cm^2) which was removed after 20 min. This application method was the same as that used in our previous study [10] and different from that reported by Lee et al. [8]. We also applied 10 m*M* magnesium chloride solution using another method as reported Lee et al. [8]. Immediately after tape stripping, the flank of the hairless mice was submerged in the solution or pure water for 2 h. TEWL was measured again 3, 6 and 9 h after barrier disruption. The percent recovery was calculated using the formula: [1–(TEWL immediately after treatment–TEWL at the indicated time)/(TEWL immediately after treatment–baseline TEWL)] × 100%.

Statistics

Data are presented as mean values ± SD. Statistical significance was determined by analysis of variance and *P*-values were calculated from Fisher's protected least significant difference (Fisher's PLSD) test.

Results

The application of 10 m*M* calcium chloride solution delayed barrier recovery (Fig. 1a). This is in agreement with the previous study by Lee et al. [8]. On the other hand, the

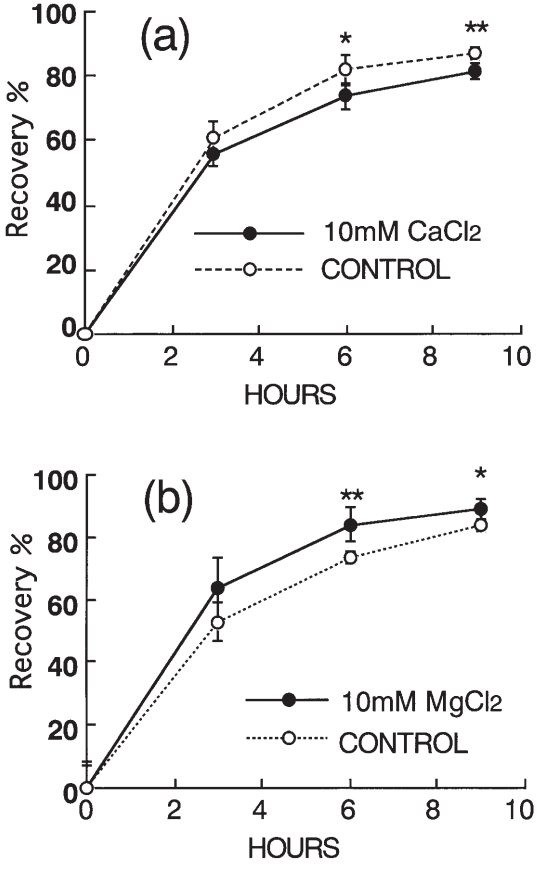

Fig. 1 a, b The effects of calcium and magnesium chloride salts on barrier recovery. Aqueous solution of each salt was spread on the flank skin of hairless mice, occluded for 20 min and then left on the skin surface to dry. The application of 10 m*M* calcium chloride aqueous solution delayed the skin barrier recovery after barrier disruption (**a**), whereas application of 10 m*M* magnesium chloride accelerated it (**b**) (n = 4, * P < 0.05, ** P < 0.01)

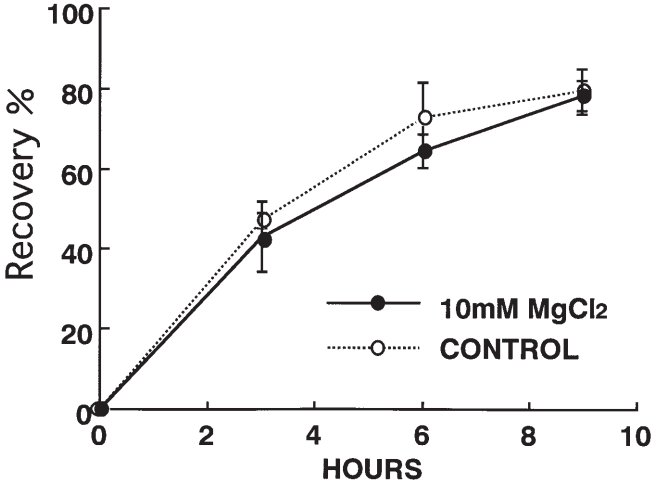

Fig. 2 The effcet of magnesium chloride salt on barrier recovery when the animal's flank was submerged in the solution or water as a control for 2 h. The submersion in magnesium chloride solution resulted slightly slower barrier recovery than submersion in water, but the difference was not statistically significant (n = 6)

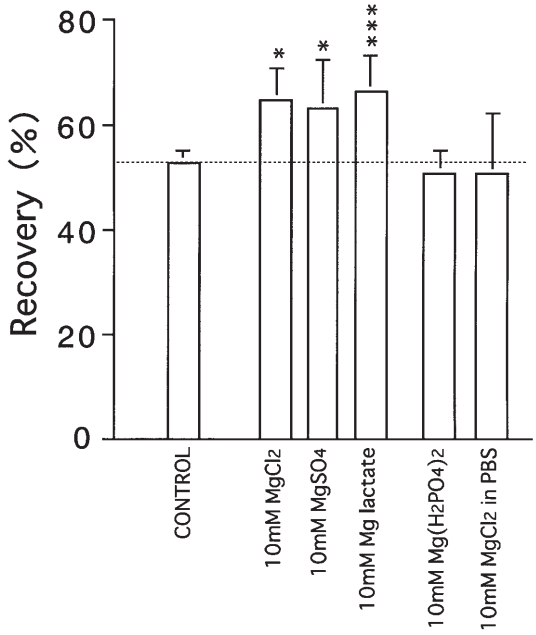

Fig. 3 The effects of various magnesium salts (10 m*M*) on barrier recovery. Magnesium chloride, magnesium sulfate, and magnesium lactate solutions accelerated barrier recovery 3 h after tape stripping, whereas magnesium bis(dihydrophosphate) and magnesiun chloride in PBS buffer did not accelerate barrier recovery (n = 6, * P < 0.05, *** P < 0.005)

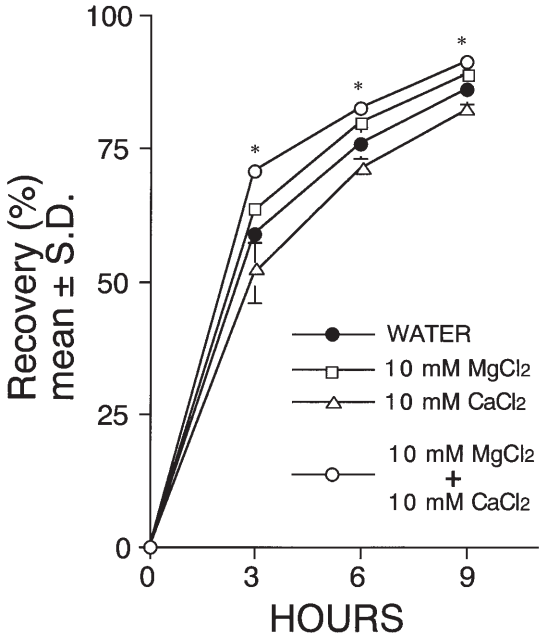

Fig. 4 The effects of calcium and magnesium chloride salts in an equimolar mixture. The equimolar (10 m*M*) mixture of magnesium chloride and calcium chloride accelerated the barrier recovery more than the 10 m*M* magnesium chloride solution ($n = 6$, * $P < 0.05$)

application of 10 m*M* magnesium chloride aqueous solution accelerated barrier recovery significantly. Figure 2 shows the effect of magnesium chloride solution on barrier recovery when the animal's flank skin was submerged in the solution or water for 2 h. In this case, magnesium chloride solution did not accelerate, or even slightly delay, barrier repair in comparison with the water-submerged control. Figure 3 shows the effect of various magnesium salts on barrier recovery. The application of magnesium bis(dihydrogen phosphate) and magnesium chloride in PBS slightly (though not significantly) delayed barrier recovery, but other salts accelerated recovery significantly. An equimolar mixture of 10 m*M* magnesium chloride and calcium chloride accelerated barrier recovery more than 10 m*M* magnesium chloride alone (Fig. 4). Figure 5 shows the effect of solutions containing magnesium chloride and calcium chloride at various ratios. The amount of total salt was kept at 10 m*M* in each solution. When the molar ratio of magnesium chloride to calcium chloride was 1 or greater, the rate of barrier recovery was significantly higher than that obtained in the water-treated control group.

Discussion

Calcium ion appears to play a crucial role in cutaneous barrier homeostasis [11], and also in the development of the skin barrier [5]. Lee et al. [8] demonstrated that the topical application of calcium ion delays barrier recovery. This is in agreement with our present study. However, they also reported that magnesium ion modestly delays barrier recovery. This discrepancy may have been caused by the difference in the application procedure. First, they used a PBS or sucrose solution as the vehicle for magnesium chloride, but we used each magnesium salt solution only and at higher concentrations. In the present study, magnesium bis(dihydrogen phosphate) and magnesium chloride in PBS also slightly delayed barrier recovery, indicating that phosphate may delay barrier recovery. Next, Lee et al. submerged the animal's flank in the solution for 2.5 h. On the other hand, we applied the solution to the skin surface, occluded it for 20 min and then left the skin surface to dry. This difference in the method of application might result in differences in the distribution of each ion. As shown in Fig. 2, submersion of an animal's flank in magnesium chloride solution did not accelerate barrier repair. The present results and also those of Lee et al. [8] indicate that the effects of magnesium ion on barrier homeostasis is dependent on the type of counter ion or other conditions which alter the magnesium distribution.

The aim of the present study was to evaluate the effect of each ion on skin barrier homeostasis and thus we did not buffer or neutralize each solution. Mauro et al. [12]

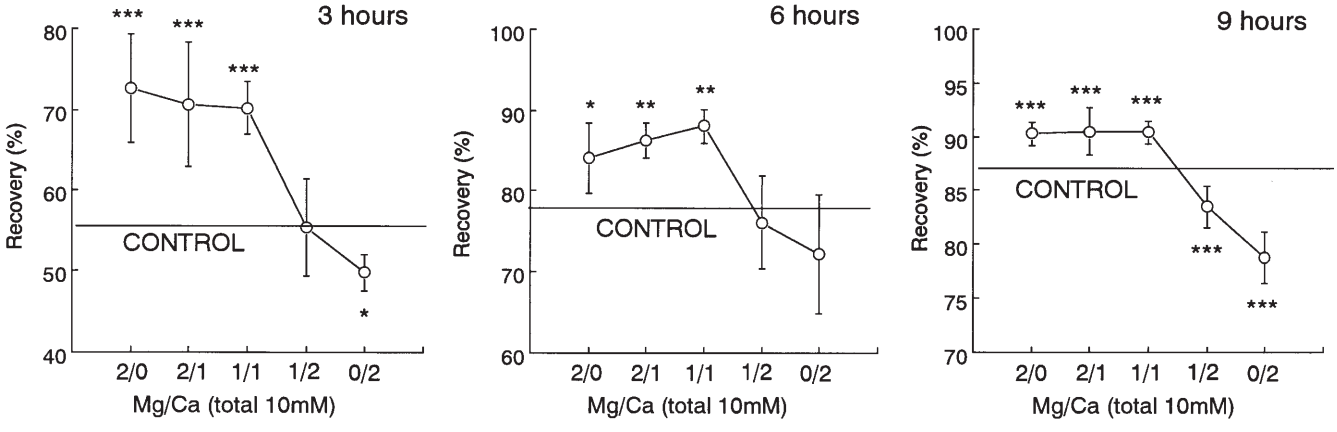

Fig. 5 The effects of calcium and magnesium chloride salts at various molar ratios. When the molar ratio of magnesium chloride to calcium chloride was greater than 1, the barrier recovery rate was significantly higher than that of the water-treated control. The total concentration of magnesium chloride plus calcium chloride was kept at 10 m*M* ($n = 6$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

have demonstrated that the barrier recovery rate is influenced by pH. We determined the pH value of each solution. Solutions of magnesium chloride, magnesium sulfate,and calcium chloride all at a concentration of 10 m*M* showed neutral pH values (7.5, 7.5 and 6.7). Thus, we could compare the effect of these solution with that of pure water. Solutions of magnesium bis(dihydrogen phosphate) and magnesium lactate showed acidic pH values (4.4 and 5.7). Mauro et al. [12] suggested that barrier repair is faster under acidic conditions than that under neutral condition. Thus, the effect of magnesium lactate solution on barrier recovery might partially be due to its acidic pH. On the other hand, in spite of its lower pH, magnesium bis(dihydrogen phosphate) solution delayed barrier repair. This result also suggests the negative effect of phosphate on barrier repair.

The role of magnesium ion on cutaneous barrier homeostasis has not been clarified yet. Zglinicki et al. have reported that magnesium concentrations are higher in the SC than other lower portions of the epidermis [7]. This suggests that magnesium ion plays a role in epidermal functions such as barrier homeostasis and epidermal differentiation. Rab-geranylgeranyltransferase, which catalyzes a posttranslational lipid modification of Rab protein requires magnesium for its activity [13]. The sequence of this enzyme is located just upstream of transglutaminase 1 on the DNA from human keratinocytes and may be related to the terminal differentiation of keratinocytes [14]. The modified Rab protein might play a crucial role in endocytosis like lamellar secretion [15]. Further work is needed on the role of these factors.

The present study produced another paradoxical result: the effect of the magnesium and calcium salt mixture. As demonstrated by Lee et al. [8] and in the present study, calcium application delays barrier recovery, but the effect of the 10 m*M* calcium chloride and 10 m*M* magnesium chloride mixture on skin barrier homeostasis was more obvious than that of the 10 m*M* magnesium chloride solution. Mauro et al. have demonstrated a decrease in calcium concentration in the upper epidermis immediately after barrier disruption [3]. Calcium ion is very important not only for terminal differentiation but also for lipid synthesis during differentiation [16]. Menon et al. [6] suggested that the low concentration of calcium ion in the upper epidermis also affects barrier homeostasis. The level of hydration of magnesium ion is stronger than that of calcium ion [17]. Thus when these ions are applied together to the skin surface, the levels of penetration of each ion might be different. This difference might preserve a better condition for skin barrier homeostasis. The physicochemical features of these ions in the epidermis should also be investigated together with their biochemical features.

Acceleration of the barrier function would be an effective method for skin care. We have previously reported [10] that trans-aminomethyl cyclohexane carboxylic acid (t-AMCHA) accelerates barrier recovery and also that the application of this reagent prevents epidermal hyperplasia. The present study suggests another new strategy for decreasing the pathology associated with cutaneous barrier abrogation.

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