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IN VITRO REPAIRABILITY FOR THE DISORDERED SKIN BY PYRROLIDONE-CARBOXYLATE SODIUM

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ABSTRACT

The excised skin of nude mice and the porcine stratum corneum pretreated with vitamin C, oleic acid or DMSO were examined by Micro FTIR spectroscopy. The C-H stretching vibrational peaks near 2920 cm^{-1} (asymmetric CH_2) and 2850 cm^{-1} (symmetric CH_2) shifted to higher wavenumber due to the lipid disorder after in vitro treatment with above enhancers. These higher wavenumbers of CH_2 stretching bands decreased gradually to the lower region after co-treatment or post-treatment with pyrrolidone-carboxylate sodium (PCA Na), dependent on the PCA Na concentration and the time of treatment. When PCA Na coexisted with vitamin C in cell compartment, the competition effect between PCA Na and vitamin C was observed. The oleic acid inserted into the lipid structure to make a larger spectral shift of both stretching bands but less repairing ability after PCA Na treatment. On the other hand, DMSO only displaced the bound protein water and slightly loosened the lipid structure to result in narrower spectral shift of stretching bands and easier and better repairing after PCA Na application.

INTRODUCTION

Recently, transdermal delivery has become as an important means of drug administration, and how to enhance the drug absorption through skin constitutes an interesting challenge to pharmaceutical scientists. The penetration enhancers are recommended to incorporate into the transdermal delivery system to improve the drug absorption (1). It is well-known that the major resistance to penetration and permeation of skin is the stratum corneum (SC). This coherent membrane consists of blocks of cytoplasmic protein matrixes (keratins) embedded in extracelluar lipid. Various pathways of drug penetration have been associated with both keratins (transcelluar route) and lipids (intercellular route) in the SC (2,3). The structure of the lipids in the SC reflected by the layered structure has been proved as a key factor in the transport barrier (4). Alteration of lipid composition or perturbation of lipid bilayered structure induced by penetration enhancers could be expected to alter the physico-chemical properties and the degree of order of the intercelluar lamellae, and thereby influence the barrier function. Although the disordered lipid structure in SC after treatment with penetration enhancers might naturally be regulated or repaired in vivo, the in vitro restorative behavior of this disordered structure has not been found.

Pyrrolidone carboxylate sodium (PCA Na) is a composition of natural moisturizing factors (NMF) which exist in normal skin (5). It has an excellent hygroscopicity and moisture-retaining ability and provides a good moisturizing effect on the skin. Although there is some doubt as to the effectiveness of PCA Na as a skin

penetration enhancer, various analogs such as 2-pyrrolidone and N-methyl-2-pyrrolidone have been proved in this respect (6,7). So far, the other functions of PCA Na, except the above water-binding capacity, are still unknown.

In the present study, the excised skins of nude mice or porcine SC were pretreated with three penetration enhancers, vitamin C, oleic acid and DMSO, respectively. Micro-FTIR spectroscopy was used to investigate the IR spectral change of lipid acyl bands in the excised skin. The effect of PCA Na on the re-ordering and fluidity of the lipid layered structure of SC caused by the above penetration enhancers was evaluated.

MATERIALS AND METHODS

Materials:

Pyrrolidone carboxylate sodium (PCA Na, Ajinomoto Co., Inc., Tokyo, Japan) was used: Ajidew is an aqueous solution containing 50% PCA Na, whereas Prodew is formulated PCA Na with other components (L-proline, saccharide, sodium lactate and protein hydrolyzate). Penetration enhancers, Vitamin C (Sigma Chem. Co., USA, Lot No. 58F-0038), dimethyl sulfoxide (DMSO, Sigma Chem. Co., USA) and oleic acid (Nacalai Tesque Inc., Kyoto, Japan) were utilized. All the other reagents were of analytical reagent grade. Double distilled water was used.

Competition study for PCA Na with vitamin C:

A fluid/fluid diffusion cell was used in this study. The excised skin of nude mice was carefully mounted on a two-chamber diffusion cell having an available diffusion area of 2.27 cm^2 and a half-cell volume of 15.0 ml (8). The temperature of cell sets was maintained at 32 °C. Five percentages of vitamin C in pH 7.4 buffer

solution was put into donor cell, and receptor cell was filled with drug-free pH 7.4 buffer solution. PCA Na (Ajidew, 1.5%) in either cell or both cells was incorporated to compete with vitamin C. Moreover, different PCA Na concentrations (1.5, 3.0, 5.0%) in donor cell affecting the competition behavior of vitamin C was also studied. After 24 hours, the IR spectral changes for all the excised skins used were determined.

Fourier transform infrared spectroscopy:

IR spectra of the excised skin of nude mice used in this experiment were obtained using an Micro-FTIR spectrophotometer equipped with an MCT detector and a zinc selenide ATR prism (Micro FTIR-200, Jasco Co., Japan). The spectra were taken at 4 cm^{-1} resolution and generally 200 scans were accumulated to get a reasonable single-to-noise ratio (9). All spectra were taken at ambient temperature (23 °C) and humidity (65% RH).

Preparation of porcine stratum corneum:

Stratum corneum (SC) of pig was prepared from its excised skin by heat separation (60 °C, 1 min) and then by digestion in 0.5% trypsin solution (pH 7.4 tris-buffer solution) for 3 hours. The SC was then isolated and air-dried on a filter paper at room temperature and stored at desicator for further use (8).

Pre-incubation with oleic acid or DMSO:

Several pieces of porcine SC were incubated with oleic acid (5% in propylene glycol) or DMSO. At the prescribed interval, one piece of SC was removed and rinsed with distilled water (containing 50% ethyl alcohol) several times, then dried in a dessicator. Each SC was examined by Micro FTIR spectroscopy. At the end of the incubation period, the remained SC was rinsed with distilled water and then re-immersed in

2.5% of PCA Na (Ajidew or Prodew) aqueous solution. IR spectra were determined by Micro FTIR photometer at the prescribed intervals. The experiments were performed in triplicate at ambient temperature.

RESULTS AND DISCUSSION

Lipid bilayers in an ordered gel state often exhibit its IR absorption spectra of lipid acyl chains at lower frequencies than lipids in a disordered liquid crystalline state. Among many IR spectral changes, the frequency of the CH_2 stretching bands near 2850 and 2920 cm^{-1} becomes higher .pawhen the degree of disorder of the lipid acyl chains increases (10).

Figure 1 shows the IR spectra, ranging from 3000 to 2600 cm^{-1}, of the excised skin of nude mice after different treatments. The IR spectra of the C-H symmetric (near 2850 cm^{-1}) and asymmetric (near 2920 cm^{-1}) stretching vibrational peaks of the intact skin without any treatment were 2850.17 and 2918.65 cm^{-1}, respectively. Once the skin was treated with vitamin C, however, both spectra shifted to 2851.94 and 2921.31 cm^{-1}. Obviously, treatment with vitamin C might cause a blue shift of approximately + 2.66 cm^{-1} for asymmetric C-H band and + 1.77 cm^{-1} for symmetric C-H band. Since the C-H asymmetric stretching absorbance near 2920 cm^{-1} is more sensitive than that near 2850 cm^{-1} in the lipid layer (11), thus the asymmetric C-H bands for the excised skin of nude mice after treatment with vitamin C shifted apparently to higher wavenumber. It has been reported that vitamin C can partly cleave the disulfide bonds of protein, increase the hydration capacity of the protein, and finally increase the drug penetration (12). This cleavage of disulfide bonds in skin protein can somewhat disorder the lipid-bilayer structure by

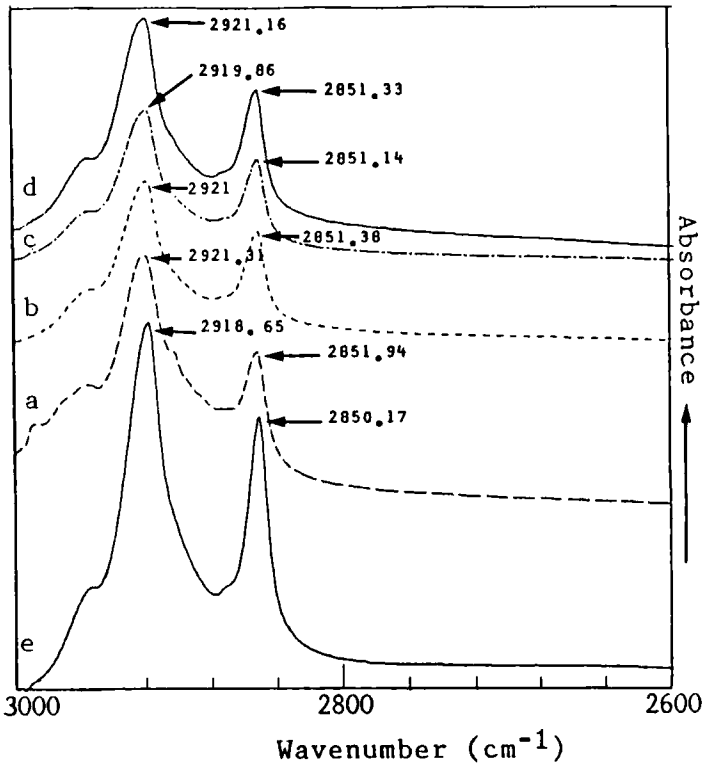

FIGURE 1

The IR spectral changes of the excised skin of nude mice after treatment with vitamin C and/or PCA Na in the cell compartment
Key:
Vitamin C was existed in donor cell
PCA Na (Ajidew, 1.5%) was added in:
b, receptor cell; c, donor cell; d, both cells;
a, without PCA Na; e, normal skin without any treatment

conformational change, leading to a little spectral shift of C-H stretching peaks. However, both higher wavenumbers of stretching bands caused by vitamin C decreased somewhat after PCA Na came to coexist with vitamin C in cell compartment.

Figure 2 indicates the change in wavenumber of C-H stretching vibrational bands as an increase function of

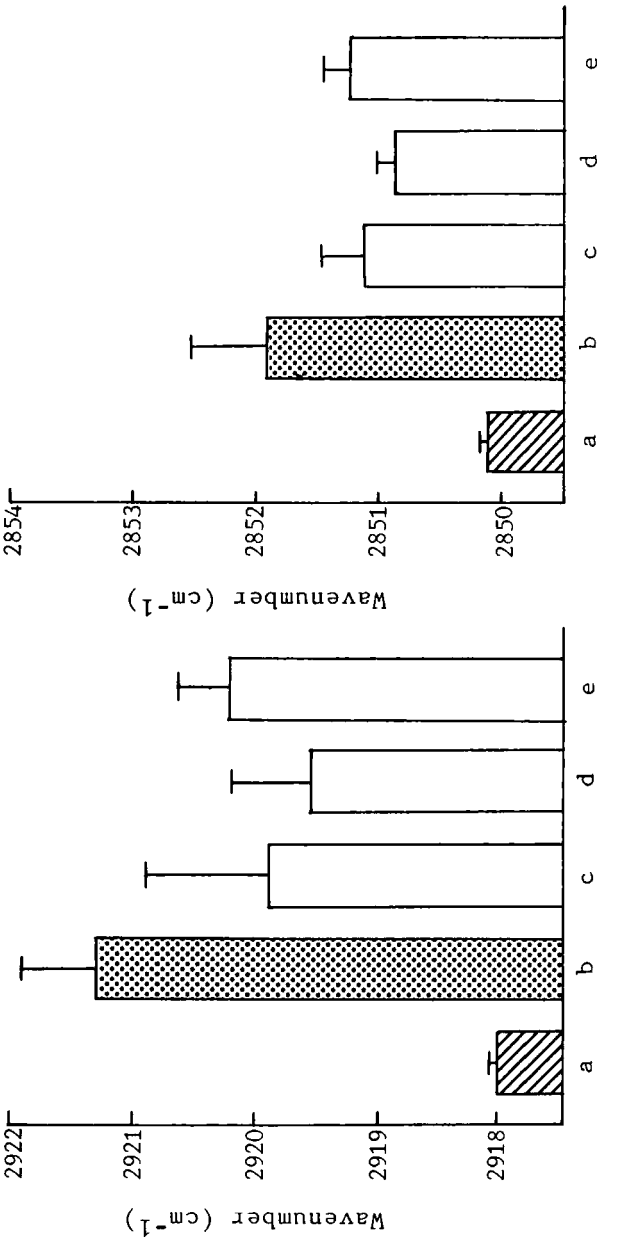

FIGURE 2

Spectral shift of lipid stretching bands of nude mice skin after pre-treatment with vitamin C and post-treatment with PCA Na

Key:

a: normal skin without any treatment
b: treatment with vitamin C for 24 hours
c-e: post-treatment with PCA Na after vitamin C pre-treatment (b)
c: Conc. of PCA Na (Ajidew); 1.5%
d: Conc. of PCA Na (Ajidew); 3.0%
e: Conc. of PCA Na (Ajidew); 5.0%

PCA Na concentration. Interestingly, the higher frequencies of C-H stretching bonds induced by vitamin C gradually shifted to lower region when different concentration of PCA Na came to coexist in vitamin C donor solution. The decrease in C-H stretching bands from higher wavenumber to lower region was found with the increase of PCA Na concentration, suggesting .pathat the competitive effect between vitamin C and PCA Na might occur with lipid disorder-order transition, and lipid-layering regulation function of PCA Na might be responsible for this result. Vitamin C can disorder the lipid bilayer structure whereas PCA Na can compete with vitamin C to prevent the formation of lipid-disordering structure. The above results strongly suggest that PCA Na can regulate the lipid-disordering bilayer structure of the exised skin of nude mice induced by vitamin C, in vitro. We also found the PCA Na-dependent changes in the peak position of scissoring vibrational bands in skin lipids. After treatment of vitamin C, the scissoring bands at 1473 and 1464 cm^{-1} diminished and flattened due to the disorder of lipid. This disappeared peaks became sharpen again with addition of PCA Na, impling that PCA Na could be used as a lipid-ordering repairer. It also confirms that PCA Na is a competitor of vitamin C and has lipid-ordering regulation function.

The effect of oleic acid and PCA Na on the IR absorption spectra of C-H stretching bands of porcine SC is shown in Fig. 3. Apparently, the C-H stretching bands of porcine SC shifted to higher region with the increase of pretreatment time of oleic acid. After 24-hr pretreatment with oleic acid, the C-H symmetric stretching band near 2850.17 cm^{-1} increased to 2852.10 cm^{-1} (+ 1.93 cm^{-1}) and the C-H asymmetric stretching band

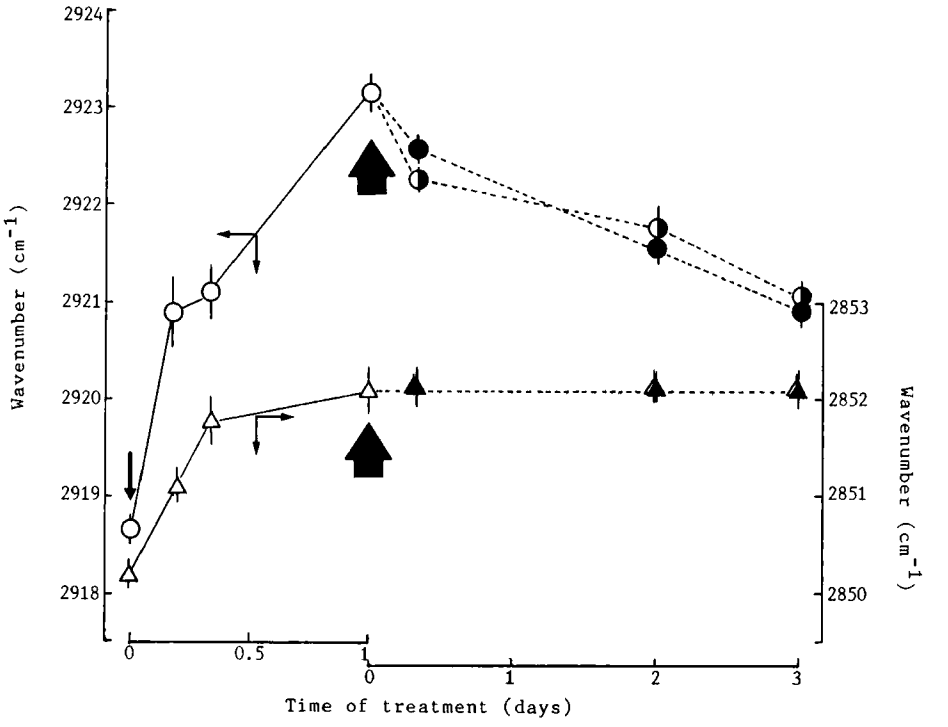

FIGURE 3

Time-dependent spectral shift of CH_2 stretching bands of porcine stratum coeneum pre-induced by oleic acid and its repairing ability after post-treatment with PCA Na
Key:
Solid line: pre-induced by oleic acid
Dotted line: post-treatment with PCA Na
●, ▲: Ajidew; ◑, ◭: Prodew

near 2918.65 cm^{-1} also shifted to 2923.16 cm^{-1} (+ 4.51 cm^{-1}), respectively. Again, asymmetric CH band showed to be more sensitive than symmetric C-H band. Since oleic acid interacts only with the SC lipids, its bent structure can penetrate into the lipid structure to disrupt the lipid-bilayer and to increase the fluidity of the lipid region (2). This dramatic change suggests that oleic acid is a strong penetration enhancer (13).

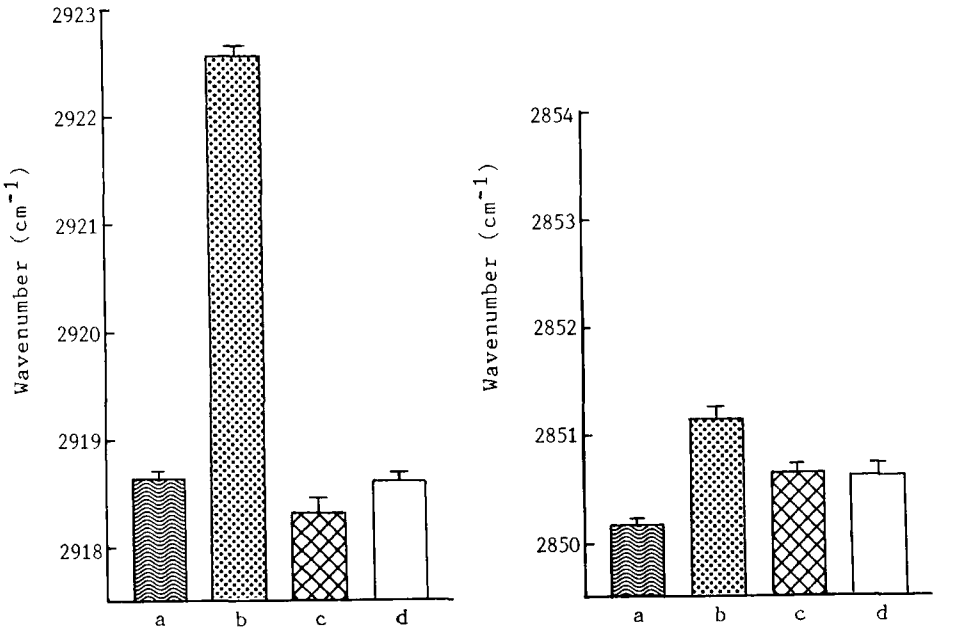

FIGURE 4

The spectral shift of CH_2 stretching bands of porcine stratum corneum pre-induced by DMSO and its repairing ability after post-treatment with PCA Na
Key:
a, normal SC; b, pre-treatment with DMSO for 24hrs; c and d, post-treatment with Prodew and Ajidew

The C-H asymmetric stretching band of porcine SC at 2923.16 cm^{-1} was gradually repaired to lower region with the immersion time of PCA Na (Ajidew or Prodew); however, the C-H symmetric stretching band at 2852.10 cm^{-1} did not change with the PCA Na treatment. This was probably due to the bent structure of oleic acid that inserted into the lipid structure and disrupted the bilayer structure, which made removal difficult and PCA Na unable to restore the lipid structure in a short period of time. Moreover, rigid structure of the

symmetric stretching band might also be responsible for this result (14). We also found that both Ajidew and Prodew indicated a similar effect on the C-H stretching peaks, although Prodew has many components of NMF.

When the porcine SC was pretreated with DMSO for 24 hrs, both C-H stretching bonds at 2918.65 cm^{-1} and 2850.17 cm^{-1} increased to 2922.51 cm^{-1} (+ 3.86 cm^{-1}) and 2851.14 cm^{-1} (+ 0.97 cm^{-1}), respectively, as shown in Fig. 4. DMSO is supposed not to partition directly into the lipid chains, at least not in great amount. It only displaces water from the lipid head groups and creates a larger solvation shell around these groups (2,15). This larger shell loosens the lipid packing, leading to the spectral shift of stretching C-H vibrational peaks of SC lipid. Once the porcine SC (pretreated with DMSO for 24 hrs) was re-immersed in PCA Na solution for 12 hr, its C-H stretching bands at 2922.51 and 2851.14 cm^{-1} were restored to 2918.65 and 2850.64 cm^{-1} after treatment with Ajidew; or to 2918.33 and 2850.68 cm^{-1} after treatment with Prodew. Moreover, a significant difference between 1500-1200 cm^{-1} for intack porcine SC and SC pretreated with DMSO also appeared, but it was repaired to similize the original IR spectra of intack porcine SC when Ajidew or Prodew was post-treated. This suggests again that Ajidew and Prodew could repair the deformed protein and lipid in disordered porcine SC.

In conclusion, it will be of interest to find that PCA Na has an in vitro repairable function to restore the disordered structure of lipid and protein of the excised skin pretreated with penetration enhancers. This repairing ability might be deduced that PCA Na is a component of natural moisturizing factor (NMF), which

has previously penetrated into skin and absorbed water to re-arrange the disordered lipid bilayer structure.

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