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Physicochemical properties of macrogol ointment and emulsion ointment blend developed for regulation of water absorption☆

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ABSTRACT

Pressure ulcers can form with excess pressure and shearing stress on skin tissue. Because pressure ulcer is often accompanies by exudates, selection of appropriate topical emulsion ointment is difficult. Blended ointments consisting of emulsion base and water-soluble base are clinically used for adjustment of wound moist environment. Because regulating the amount of wound exudates can enhance treatment efficacy, two new blended ointments were developed. LY–SL blended ointment consisted of lysozyme hydrochloride water-in-oil (w/o) emulsion (LY-cream) and sulfadiazine macrogol (polyethylene glycol) ointment (SL-pasta). TR–SL blended ointment consisted of tretinoin tocoferil oil-in-water (o/w) emulsion (TR-cream) and SL-pasta (TR–SL). LY–SL and TR–SL were applied to Franz diffusion cell with cellulose membranes for the evaluation of water absorption characteristics at 32 °C. Water absorption rate constants ($mg/cm^2/min^{0.5}$) were 12.5, 16.3 and 34.6 for LY-cream, TR-cream and SL-pasta, respectively. Water absorption rate constants for LY–SL and TR–SL (SL-pasta 70%) exhibited intermediate values of 21.2 and 27.2, as compared to each ointment alone, respectively. Because amount of water absorbed was linearly related to square root of time, it was suggested that water-absorbable macrogol was surrounded by oily ingredients forming matrix structure. This diffusion-limited structure may regulate water absorption capacity. This is the first report of physicochemical properties of macrogol ointment and emulsion ointment blend developed for regulation of water absorption. The blended ointment can properly regulate amount of exudates in wounds and may be useful for treatment of pressure ulcers.

1. Introduction

Proper moist environment can promote wound healing. Wound exudates contribute to moist environment. Water-soluble base having water absorption property is used for wounds rich in exudates and emulsion base having water-retaining property is used for wounds poor in exudates (Miyachi, 2009). Use of emulsion base having water-retaining property for wounds rich in exudates can induce excessively moist environment. Conversely, use of water-soluble base for wounds poor in exudates can induce excessively dry environment. Both procedures can retard healing processes. Topical products often used in Japan for treatment of pressure ulcers include LY-cream (Reflap™), TR-cream (Olcenon™) and SL-pasta (Teradia Pasta™). LY-cream is a water-in-oil (w/o) emulsion containing lysozyme hydrochloride (LY) (Yamamoto et al., 1996) and is recommended for promoting granulation tissue in skin ulcers including pressure ulcers. TR-cream is an oil-in-water (o/w) emulsion containing tretinoin tocoferil (TR) (Kawabata et al., 2002). SL-pasta is a macrogol (polyethylene glycol) ointment (MO) containing sulfadiazine (SL) used for treatment of wounds rich in exudates. When emulsion base is used for wounds rich in exudates, one cannot control the amount of exudates. When emulsion base with granulation tissue promoting is blended with SL-pasta with MO base for the treatment of wounds rich in exudates, the blended ointment can properly regulate amount of exudates in wounds, greatly enhancing treatment efficacy (expert opinion, Mizokami et al., 2010).

We have previously established the evaluation method of water absorption capacity of ointment base by using Franz cell model with semi-permeable membranes (Noda et al., 2009). Using this method we have previously reported that water absorption capacity of ointment base is classified based on the mode of absorption. One is an active type, where base can absorb water by osmotic pressure. The

Abbreviations: TR, tretinoin tocoferil; LY, lysozyme hydrochloride; SL, sulfadiazine; BSA, bovine serum albumin; MWCO, molecular weight cut-off; MO, macrogol ointment.

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other is a passive type, where base can absorb water into matrix by diffusion-control (Noda and Fujii, 2010). Using this method we aimed to determine the water absorption characteristics of blended ointments consisting of water-soluble base and emulsion base.

2. Materials and methods

2.1. Materials

LY cream was from Eisai Co., Ltd. (Tokyo, Japan). TR cream was obtained from Kyorin Rimedio Co., Ltd. (Kanazawa, Japan). SL-pasta was from Daiichi Sankyo Co., Ltd. (Tokyo, Japan). These ointments are available in Japan. Components of w/o emulsion base for LY-cream are liquid paraffin, white petrolatum, white beeswax, cetyl palmitate, octyldodecanol, cetanol, stearyl alcohol, paraffin, sorbitan monostearate, polyoxyethylene cetyl ether, polyoxyl (40) stearate, aluminum stearate and D-sorbitol. Components of o/w emulsion base for TR-cream are liquid paraffin, cetanol, polyethylene glycol monostearate, isopropyl myristate, glycerin and D-sorbitol. Components of water soluble base for SL-pasta are macrogol 400 (molecular weight about 400) and macrogol 4000 (molecular weight about 4000). MO is generally mixture of equal amount of macrogol 400 and macrogol 4000. The mixing ratio of other components is not disclosed. Bovine serum albumin (BSA) and cellulose ester membrane were from Wako Pure Chemical Co., Ltd. (Osaka, Japan). The phosphate buffered saline (PBS) was prepared by Mg/Ca ion free Dulbecco's prescription. The simulated wound exudates supplemented with 5% BSA was prepared by Hanks' prescription.

2.2. Methods

2.2.1. Measurement of water absorption rate using Franz diffusion cell

LY cream or TR cream was tempered with appropriate quantity of SL-pasta on a plate to give the blended ointment with contents of SL-pasta adjusted between 0 and 100% on the basis of the total weight. Uniform mixture of the components was achieved by ointment slab and spatula and was visually confirmed. The blended ointment sample (1.2 g) was applied to the cellulose membrane mounted on the Franz diffusion cell (Kawashima et al., 1993; Noda et al., 2009). Twenty mL of simulated wound exudates was introduced to the bellower cell. Molecular weight cut-off (MWCO) values of the cellulose ester membranes used were 100 kDa (Spectrum Laboratories, CA). A water jacket of the permeation cell maintained the system at 32 °C. The temperature was maintained at 32 °C from the respective of the OECD guidance document for the conduct of skin absorption studies. After every 30 min the water level in the branch tube attached to the cell was checked and the simulated fluid was added to the cell from the edge of the branch tube by a syringe until the water level reached its original level. The reduction of syringe weight by adding the simulated fluid was considered equivalent to amount of water absorbed to the ointment sample. Measurements were performed at least 3 times and the means of amount of water absorbed were calculated.

2.2.2. Quantitative analysis of ointment phase separation by ultracentrifugation

Five g of the blended ointments were centrifuged at 25 °C at 16,000 rpm for 1 h by the ultracentrifuge equipment with angle rotor (XL-90, Beckman Coulter, Tokyo, Japan: Vold and Mittal, 1972; Okamoto and Oishi, 1977). The aqueous layer was then collected with a syringe and amount of the aqueous layer was weighed to give the separation ratio of aqueous phase on the basis of the total weight.

2.2.3. Microscopic observation of the dispersed system of blended ointments

The blended ointment was spread out on the glass slides with cover glass. The dispersed system was observed by phase contrast microscopy (IX41, Olympus, Japan).

2.2.4. Assessment of the stability of medicinal properties in the blended ointments

After preparation 10 g of the blended ointments were stored in an ointment container at 25 °C under the dark. Samples were collected at 0, 2, 4 and 24-weeks for determination of the concentration of intact medicinal properties in the blended ointments. The concentration of TR and SL were determined by HPLC analysis. As to LY-cream the residual activities of LY in the blended ointments were assessed by bacterolytic activities against *Micrococcus luteus*. The conditions of HPLC analysis and bacterolytic assay were in accordance with the Japanese Pharmacopeia 14th edition.

2.3. Data analysis

All experiments were performed at least in triplicate. Data are expressed as means ± standard deviations (SDs). Water absorption rate constants were obtained from the slope of the regression line. The differences in the water absorption rate constants of blended ointments were evaluated using Tukey's multiple comparison tests. Probability values of less than 0.05 were considered statistically significant.

3. Results

3.1. Water absorption property of blended ointments used to absorb simulated wound exudates

LY–SL, a blend of LY-cream and SL-pasta, could be prepared in a reproducible fashion except when combination ratio of SL-pasta was 20%, where ointments were separated and could not be prepared.

TR–SL, a blend of TR-cream and SL-pasta, could also be prepared in a reproducible fashion. When combination ratio of SL-pasta was 100%, cumulative amount of water absorbed increased linearly with time and the ointment was completely dissolved in 1 h. When combination ratio of SL-pasta was decreased, ointment was not completely dissolved at 1 h. When amount of water absorbed was plotted against time, the amount of water absorbed per unit of time was reduced. When amount of water absorbed was plotted against the square root of time, a linear correlation was observed at any combination ratio (Fig. 1). If Fickian diffusion is the predominant mechanism of this process, plots of the initial amount of water absorption versus the square root of time should deliver a straight line (Peppas, 1985). Plots of the initial amount of water absorption versus time delivered a curved line, indicating that the rate of water absorption decreases over time. Thus, the water absorption rate constants were obtained from the slope of the regression line of Fig. 1 and used for the comparison of the alterations of water absorbing capacity. The slope of the line became shallow as the ratio of SL-pasta became smaller. The water absorption rate constants of LY-cream, TR-cream and SL-pasta were 12.5, 16.3 and 34.6 ($mg/cm^2/min^{0.5}$), respectively when they were used as single agents (Table 1). The water absorption rate constant calculated from the slope of the lines shown in Fig. 1 was plotted against the combination ratio of SL-pasta (%) (Fig. 2). With LY–SL no significant changes were observed when the combination ratio of LY–SL was within a range of 0–40%, 50–80% and 90–100% (Fig. 2a). There were no significant differences between values at 40% and 50%. Significant differences were observed between values at 0% and 50% ($p < 0.01$), between values at 50% and 90% ($p < 0.01$) and between