SEARCH PAPER

In Vivo and In Vitro Evaluations of Water-Absorption Properties of Various Ointments

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ABSTRACT

The water-absorption properties of various vehicles were evaluated in rats whose stratum corneum had been stripped off over the application site. Polyethylene glycol (PEG) applied to the site effectively absorbed the exudate, but other applied ointments absorbed little exudate or water. By the PEG ointment, the exudate was absorbed continuously and extensively, and a linear relationship was obtained between the amount of water absorbed and the square root of time. To establish an acceptable alternative in vitro method for the design of a suitable ointment for treating skin ulcers with exudate, in vitro evaluations were performed by three methods: the Franz cell method, the cellulose membrane diffusion method and the spontaneous water uptake method. The membrane diffusion method, and the spontaneous water uptake method appeared to be acceptable alternatives to the in vivo method in evaluating the water absorption of ointments. It is a simple and useful method for preliminary formulation studies of ointments for the treatment of skin ulcers with exudate.

INTRODUCTION

he choice of an ointment base depends upon many ırs, such as the action desired, the nature of the me- ıent to be incorporated and its bioavailability and lity, and requisite shelf-life of the finished product The optimum ointment base should be selected according to the characteristics of the drug and of the disease states or skin conditions to be treated (2,3). For particular use in the treatment of skin ulcers with exudate, an ointment base having water absorbing and desiccant properties is preferable, and so evaluation of the water-absorption capacity of the vehicle is important. A number of methods designed to evaluate the absorption

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rate and capacity of pastes and ointments were considered in the design and development of a suitable composition. Casparis and Meyer proposed a method which involved adding water to the melted base, cooling the mixture to room temperature, and rubbing it on a slab with a spatula (4). Halpern and Zopf placed the ointment in a mortar and added the water from a burette (5), and a number of studies were performed by this method or variations (6–9). Rae reported another method which measured the passage of water through a cellulose film (10). He used a glass tube open at both ends, attached the film at one end, placed the ointment in the tube, and then immersed the tube in distilled water (11). Another method, which used a tensiometer, was reported (12,13), and Ishida et al. assessed the water absorption by keeping the ointment in an incubator for a fixed interval (14). However, no in vivo evaluation study was performed.

The objectives of the present study were to determine the water-absorption properties of various vehicles in vivo and to establish an acceptable alternative in vitro method for the design of a suitable ointment formulation. In vivo evaluation was performed in rats in which the stratum corneum of the application site had been stripped off. In vitro evaluations were performed by three methods: the Franz cell method, the cellulose membrane diffusion method, and the spontaneous water uptake method. The water-absorption profiles obtained for various ointments by these methods were compared with the results of the in vivo experiment.

MATERIALS AND METHODS

Materials

Polyethylene glycol (PEG; Nippon Oil & Fats, Tokyo, Japan), white petrolatum (Penreco, PA), cholesterol (Kishida Chemical, Osaka, Japan), stearyl alcohol (Wako Pure Chemical Industries, Osaka, Japan), white wax (Miki Chemical Industries, Hyogo, Japan), and polyethylene glycol monostearate (PGMS; Tokyo Kasei Industry, Tokyo, Japan) were used as vehicle components. The PEGs used in this study were macrogol 400 and 4000 of JP XII grade, which had average molecular weights of 380–420 and 2600–3800, respectively. Cellulose membrane was obtained from Bel-Art Products (Pequannock, NJ).

Preparation of Ointment

The ointment compositions are listed in Table 1. Formulas A to E were formulated using a mortar and pestle in the usual manner. The mixtures of PEG and petrolatum ointment (formulas F to J) were also prepared by heating the PEG (formula A) and the petrolatum (formula B ointment at 50–60°C, mixing using a mortar and pestle, and then gradually cooling the mixture while stirring continuously.

Evaluation of Water-Absorption Profile In Vivo

Eight-week-old male Sprague–Dawley strain rats were used. The animals had free access to F-2 diet (Funabashi Nojo) and water before the experiment. Their abdominal hair was shaved with an electric clipper and shaver 16 hr before the experiment. The rats were anesthetized with pentobarbital (i.p.), and the stratum corneum was removed by applying adhesive tape to the abdominal surface, and then stripping it off, 20 times. A plastic cell having an internal diameter of 3 cm and an external diameter of 4 cm was adhered to the site of application using a cyanoacrylate adhesive. One of the various ointments or 1 ml of saline was placed in the cell, the upper orifice of which was sealed with adhe-

Table 1

Formulas of Ointments for Water-Absorption Studies

	A	B	C	D	E	F	G	H	I	J
Macrogol 400	50.0			10.5	32.3	5.0	15.0	25.0	35.0	45.0
Macrogol 4000	50.0				14.0	5.0	15.0	25.0	35.0	45.0
White petrolatum		100.0	85.2	78.9	39.7	90.0	70.0	50.0	30.0	10.0
Cholesterol			3.2		3.2					
Stearyl alcohol			3.2							
White wax			8.4	5.3	5.4					
PGMS[a]				5.3	5.4					

[a]Polyethylene glycol monostearate.

sive tape. The ointment and exudate were re-covered with cotton wool at regular intervals and weighed.

In Vitro Membrane Diffusion Method

A plastic diffusion cell unit having 3 cells (diam. 3 cm) with a cellulose membrane was used. The basic design for the cell unit is illustrated in Fig. 1. Various ointments were applied to the upper side of the membrane, and the cell unit was immersed up to the level of the membrane in saline maintained at 37°C. Then, the top of each cell was immediately sealed with adhesive tape. The ointment was recovered at regular intervals and weighed.

In Vitro Franz Diffusion Cell Method

Rat skin which had been stripped in the same manner as in the in vivo evaluation was excised. The skin samples were sandwiched between the upper donor compartment and lower acceptor compartment of the homemade vertical Franz-type glass diffusion cell. The circular area of skin that was in contact with these two compartments measured 3.14 cm^2. The acceptor compartment contained 18 ml of saline solution. The temperature of the cells was maintained at 37°C, and the acceptor solution was stirred with a magnetic stirrer. One gram of ointment was placed in the donor compartment on the stripped skin, then the top of cell was sealed with adhesive tape. The ointment was recovered at regular intervals and weighed.

In Vitro Spontaneous Water Uptake Method

Various ointments were spread evenly over the entire surface of the plastic container (diam. 4.2 cm) and weighed. The container was kept at 30°C and 90% RH for 72 hr and then weighed.

RESULTS AND DISCUSSION

Water-Absorption Profile In Vivo

Figure 2 shows the effect of the amount of PEG ointment on the water-absorption profile at 2 and 8 hr after application in the rats. Water absorption rose as the amount of ointment applied increased, and reached a saturation level of approximately 0.2 g/cm^2 when more than 0.5 g of ointment was applied for 2 hr. In contrast, a saturation level of approximately 0.7 g/cm^2 was obtained when more than 1 g of ointment was applied for 8 hr. The following studies were therefore performed using a dose of 1 g.

The mean water-absorption profiles, showing the

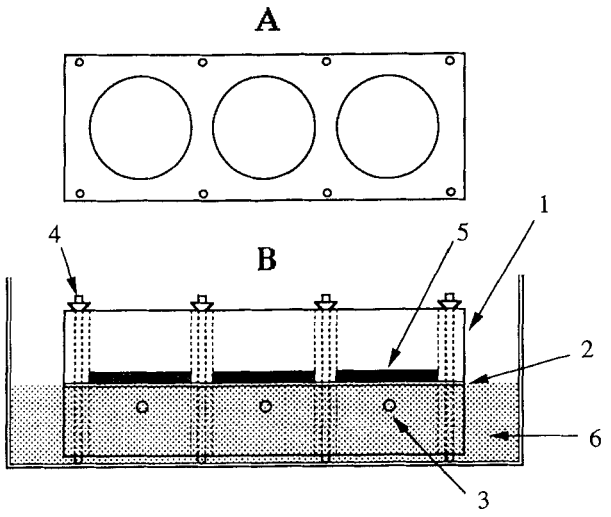

Figure 1. Schematic illustration of apparatus for evaluation of water-absorption profile in in vitro membrane diffusion method. (a) Plane figure of plastic diffusion cell. (b) Cross section of the apparatus. 1, plastic diffusion cell unit (diam. 30 mm); 2, cellulose membrane; 3, air release hole; 4, screw; 5, ointment; 6, saline solution.

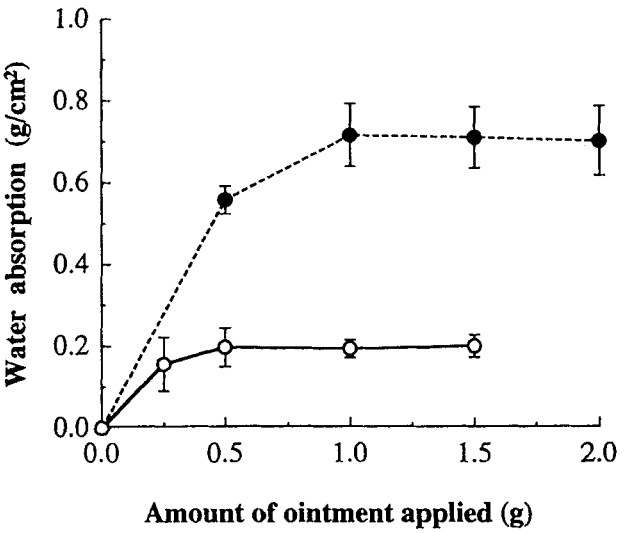

Figure 2. Effect of amount of PEG ointment on water-absorption profile in rats. Key: (○) 2 hr after the application; (●) 8 hr after the application. Each point represents the mean ± SD (n = 3-4).

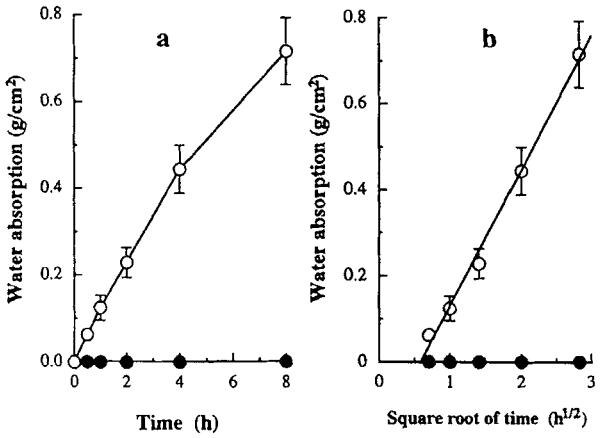

Figure 3. In vivo water-absorption profile of PEG (○) and petrolatum (●) ointment following topical application of ointment (1 g/head). (a) plots of the amount of water absorbed against time; (b) plots of the amount of water absorbed against the square root of time. Each point represents the mean ± SD (n = 3–5).

changes in the amounts per unit application area following topical application of PEG and petrolatum ointment in rats, are presented in Fig. 3. Little water absorption followed the application of petrolatum ointment. By the PEG ointment, the exudate was absorbed continuously and extensively. When the amounts of water absorbed were plotted against the square root of time, a linear relationship was obtained with PEG ointment [Fig. 3(b)]. The absorption rate of water for PEG ointment was 0.32 g/cm^2/hr$^{1/2}$, and the lag time was 0.35 hr.

The water-absorption ratios of various ointments 2 hr after application in rats are shown in Table 2. The PEG ointment (formula A), which absorbed about 1.5 times the amount of water in 2 hr, is the most absorbent of the ointments tested. The PEG ointment–petrolatum mixture (formula D), which absorbed about 25% of the water in 2 hr, exhibited a better water-absorption profile than the petrolatum–glycol emulsion (formula E), hydrophilic petrolatum ointment (formula C), and petrolatum ointment (formula B). The water-absorption ratio of the control, in which saline was used instead of ointment, at 2 hr after application was -8.2%, suggesting that no apparent water or exudate efflux had occurred but that the influx was slightly greater than the efflux. These results indicated that PEG very effectively absorbed exudate, while the other ointments absorbed only a little exudate or water.

Table 2

Water-Absorption Ratios of Various Ointments 2 hr After Application in Rats

Formula	Water-Absorption Ratio (%)
A	157.3 ± 11.1
B	1.0 ± 0.5
C	3.2 ± 1.1
D	25.0 ± 7.8
E	3.1 ± 1.1
Saline	-8.2 ± 1.7

Dose: 1 g/head. Each value represents the mean ± SD (n = 3–5).

In Vitro-In Vivo Correlation for Water-Absorption Profile

From the standpoints of animal welfare, cost reduction, and the accuracy with minimum variance, the establishment of a readily usable in vitro test is very desirable, particularly for the preliminary work in formulation studies of an ointment for the treatment of skin ulcers with exudate. Three in vitro methods were studied for the correlation of the results with those obtained in vivo. Two of these were performed to compare the water-absorption rate directly with the in vivo rate, one being used on the stripped skin and the other on the cellulose membrane. The third in vitro method was an indirect comparison of the water-absorption rate. The results of the evaluation of the water-absorption profiles of PEG ointment with these methods are shown in Table 3. As with the in vivo data, a linear relationship was obtained for PEG ointment in each method, when the amounts of water absorbed were plotted against the square root of time. In the Franz cell method, the lag time of 0.386 hr was almost equal to that obtained in vivo, but absorption rate of water, 0.23 g/cm^2/hr$^{1/2}$, was slower than the in vivo rate. The vertical Franz diffusion cell method has often been used for the evaluation of active ingredient transport from a vehicle through the skin and it is reported to be comparable to an in vivo procedure. However, this method is not suitable for evaluating water-absorption profiles, because it yields a variance as great as that seen in vivo, and the diffusion of water from the acceptor compartment through the excised skin is relatively low. This may be due to skin thickness, viability, capillary blood flow, or a combination of these. However, although the lag time was smaller than in vivo, the cellulose membrane method gave almost the same water-absorption rate as in vivo.

Figure 4 shows the in vitro water-absorption profiles obtained with the membrane diffusion method for the

Table 3

Comparison of Water Absorption of PEG Ointment Using the In Vivo Method, the In Vitro Franz Cell Method and the In Vitro Diffusion Method

Method	Water Absorption[a] (g/cm^2)	k[b] ($g/cm^2/hr^{1/2}$)	Lag Time (hr)
In vivo	0.717 ± 0.077	0.315	0.345
In vitro Franz cells	0.515 ± 0.115	0.227	0.386
Membrane diffusion	0.693 ± 0.005	0.271	0.063

[a]Amount of water-absorption quantity per unit of application area 8 hr after application. Each value represents the mean ± SD (n = 3–5).
[b]Absorption rate, determined by plotting the amounts of water absorbed against square root of time.

PEG and petrolatum ointments. Although the amount of absorption in the start was slightly greater than that seen in vivo (Fig. 3), the water-absorption profile obtained by this method closely resembled the results of the in vivo experiment. This method was therefore considered comparable with the in vivo method.

Figures 5 and 6 show the in vitro/in vivo correlation for the water-absorption profiles of various ratios of PEG and petrolatum ointment in the membrane diffusion method and the spontaneous water uptake method, respectively. Reasonable agreement was obtained between the water-absorption profiles 8 hr after application in vivo and in the in vitro membrane diffusion method. Also, although the water-absorption rates were different, the amount of water absorbed over 72 hr in the in vitro spontaneous water uptake method was almost equal to the amount of water absorbed in 8 hr in vivo. In these three methods, the water-absorption profiles of the various mixtures of PEG and petrolatum depended on their content ratio, the amount of water absorbed decreasing as the percentage of petrolatum increased. When the petrolatum content was over 50%, there was little change in water-absorption capacity. Further work is required to explain this observation.

CONCLUSION

It was confirmed that PEG is highly effective in absorbing exudate, while the other ointments examined absorb exudate or water only slightly. Two in vitro

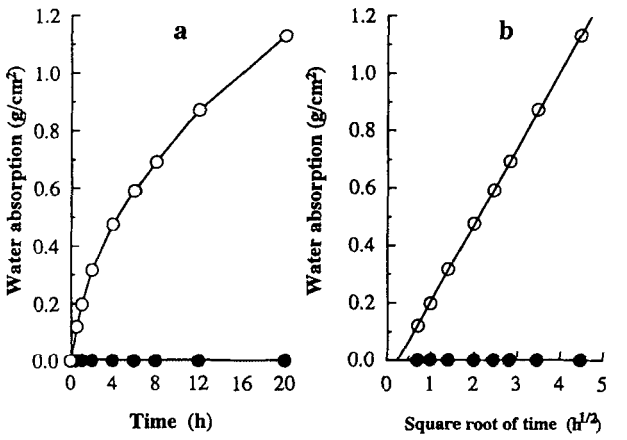

Figure 4. In vitro water-absorption profile of PEG (○) and petrolatum (●) ointment in the membrane diffusion method. (a) Plots of the amount of water absorbed against time; (b) plots of the amount of water absorbed against the square root of time. Each point represents the mean ± SD (n = 3–4).

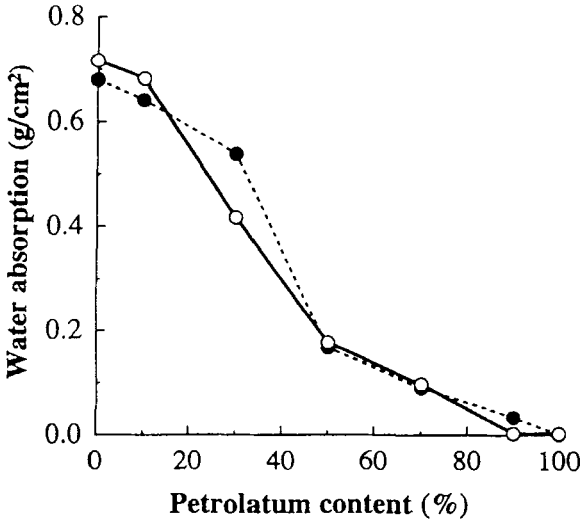

Figure 5. Comparison between in vivo method (○) and in vitro membrane diffusion method (●) for water-absorption of various mixtures of PEG and petrolatum ointments. Data are expressed as the mean for three to five experiments.

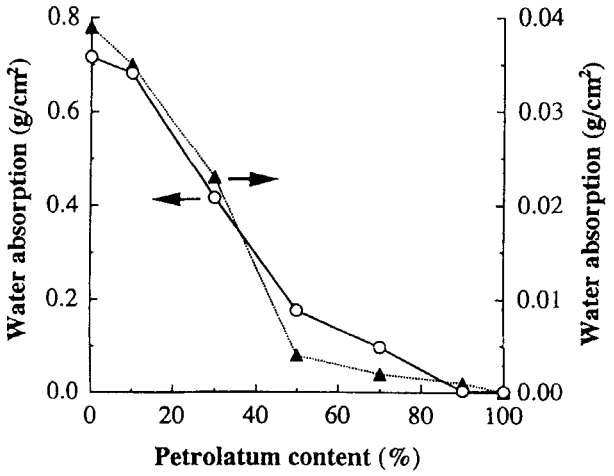

Figure 6. Comparison between in vivo method (○) and in vitro spontaneous water uptake method (▲) for water-absorption of various mixtures of PEG and petrolatum ointments. Data are expressed as the mean for three to five experiments.

methods, the membrane diffusion method and the spontaneous water uptake method, are acceptable alternatives to the in vivo technique for evaluating the water absorption of an ointment. The membrane diffusion method, in particular, is a simple and useful method for the preliminary stage of the formulation studies of ointments for treating skin ulcers with exudate.

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