Effect of Vehicles on Percutaneous Absorption of Bucladesine (Dibutyryl Cyclic AMP) in Normal and Damaged Rat Skin

Eiichi MAFUNE,^{*,a} Masayuki TAKAHASHI,^b and Norio TAKASUGI^b

Pharmaceutical Formulation Research Center,^a Drug Delivery Research Center,^b Developmental Research Laboratories, Daiichi Pharmaceutical Co., Ltd., 16-13, Kitakasai 1-chome, Edogawa-ku, Tokyo 134, Japan.

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Bucladesine, sodium *N*⁶,2'-*O*-dibutyryl cyclic 3',5' adenosine monophosphate (DBcAMP), which is effective for the treatment of chronic skin ulcers including decubitus ulcer, was evaluated for percutaneous absorption in rats with normal skin, stripped skin and full-thickness abrasion models. Percutaneous absorption from aqueous solution or ointment was very low in intact skin. When the aqueous solution was applied to the site where the skin had been excised, DBcAMP was absorbed very rapidly and almost completely. In the case of stripped skin, DBcAMP was absorbed rapidly but slower than in the full-thickness abrasion model. In both damaged skin models, a better absorption profile was obtained with the polyethylene glycol (PEG) than the petrolatum ointment and DBcAMP was released continuously from the PEG ointment, indicating that this ointment is suitable for the treatment of ulcers of the skin. The percutaneous absorption from stripped skin was considerably influenced by the powder formulation. It is appropriate to evaluate the bioavailability in damaged skin models for a drug, such as DBcAMP, which is used in the treatment of skin ulcer.

Key words percutaneous absorption; DBcAMP bioavailability; damaged skin; rat; ointment

Bucladesine, sodium *N*⁶,2'-*O*-dibutyryl cyclic 3',5' adenosine monophosphate (DBcAMP), is a derivative of cAMP which easily penetrates the cell membrane and is resistant to hydrolysis by phosphodiesterase.¹⁾ It has been reported that this drug has several actions on the cardiovascular system.²⁾ More recently, DBcAMP has been reported to be effective for the treatment of chronic skin ulcers including decubitus ulcer. Kasai *et al.* reported that a polyethylene glycol (PEG) ointment containing DBcAMP was effective for the treatment of rat skin burns.³⁾ The effects of DBcAMP on human skin ulcers were demonstrated in various clinical studies.⁴⁾

It is widely known that the formulation has considerable influence on percutaneous absorption of a topical drug. The vehicle affects any stage in percutaneous absorption of active ingredient,⁵⁾ such as drug dissolution and diffusion in the vehicle,⁶⁾ partition into stratum corneum,⁷⁾ or diffusion in the skin.⁸⁾ On the other hand, skin diseases cause alteration in skin conditions, and thus influence the percutaneous absorption.⁵⁾ In general, the absorption increases in diseases characterized by a defective stratum corneum. Therefore, it seems valuable to evaluate the bioavailability of DBcAMP in damaged skin because the drug is used for the treatment of skin ulcer.

In this study, we evaluated the absorption of DBcAMP aqueous solution in rats with normal skin, stripped skin, and full-thickness abrasion model. Stripping is widely used as an experimental method for damaging skin.⁵⁾ The full-thickness abrasion model was employed to study the skin regeneration and the wound healing process,⁹⁾ and was used to evaluate the effect of DBcAMP and related compounds.¹⁰⁾ In the stripped skin model stratum corneum was removed and in the full-thickness abrasion model epidermis and part of the dermis were removed. We also studied the effect of vehicles on absorption of DBcAMP through normal and damaged skin in rats.

MATERIALS AND METHODS

Materials DBcAMP synthesized by Daiichi Pharmaceutical Co., Ltd., was used. PEG, petrolatum, granulated sugar, light anhydrous silicic acid (aerosil), and hydroxypropyl cellulose (HPC-L) were of JP grade. Polyvinyl alcohol (PVA) was obtained from Shin-Etsu Chemical Co., Ltd. Other chemicals were of reagent grade.

Dosage Forms For the percutaneous absorption experiments, a 3% aqueous solution of DBcAMP was prepared. Each of DBcAMP, granulated sugar, HPC-L and PVA was ground by a tablet grinder and passed through an 100 mesh sieve. Two ointments, one a 3% DBcAMP PEG ointment containing PEG4000 and PEG400 and the other a 3% DBcAMP petrolatum ointment, were prepared. PEG or petrolatum was melted at 50 to 60 °C in a glass container. The molten ointment base was added to DBcAMP and mixed using a mortar and pestle, and then gradually cooled with continuous mixing to prepare 100 g of ointment.

Three 3% DBcAMP powder preparations were also formulated: PW-1 (granulated sugar+0.5% aerosil), PW-2 (granulated sugar+0.5% aerosil+20% HPC-L) and PW-3 (granulated sugar+0.5% aerosil+20% PVA). All ingredients were mixed together using a mortar and pestle.

Animals Male 8 week old Sprague-Dawley rats were used in all experiments. The animals had free access to F-2 diet (Funabashi Nojo) and water before the experiment. For the percutaneous absorption experiments, the abdominal hair of the animals was shaved with an electric clipper and shaver 16 h prior to drug application.

Intravenous Administration The rats were given a single intravenous dose of 3 mg DBcAMP as an aqueous solution. Thereafter, blood samples were collected periodically for 8 h.

* To whom correspondence should be addressed.

Percutaneous Absorption Experiment The rats were anesthetized with pentobarbital (i.p.) and fixed on a board. For the stripped skin model, the stratum corneum was removed by stripping the abdominal surface 20 times with adhesive tape. For the full-thickness abrasion model, the abdominal skin was excised with scissors. A plastic cell having an internal diameter of 3 cm and external diameter of 4 cm was affixed to a site using cyanoacrylate adhesive. DBcAMP (30 mg/head) was applied as an aqueous solution or one of various ointments or powders. The upper part of the cell was sealed with a polyethylene film. Blood samples were obtained from the jugular vein at 0.5, 1, 2, 4 and 8 h after topical application of each formulation. Each blood sample was immediately centrifuged and plasma was separated. Plasma samples were stored at -20°C until analyzed.

Determination of Plasma DBcAMP Concentrations A 0.2 ml aliquot of plasma was placed in a 1 ml test tube and mixed with 0.2 ml of 10% trichloroacetic acid by stirring with a Vortex mixer. The mixture was cooled in ice for 10 min, then centrifuged at 3000 rpm for 10 min. A 20 μl aliquot of the supernatant was collected and injected into the HPLC system.

HPLC System A Hitachi 635 liquid chromatograph equipped with a variable wavelength UV-VIS detector (Shimadzu SPD-6AV) was used. A modified stop-flow procedure was used to introduce the samples onto a Nucleosil 5C₁₈ reversed-phase column (150 $\times$ 4.0 mm i.d.). The mobile phase consisted of 20 ml of 0.1 M KH₂PO₄, 130 ml of acetonitrile and 850 ml of water. The flow-rate was held constant at 1.0 ml/min and effluent was monitored at 273 nm.

Analysis of Data The plasma concentration-time curves of DBcAMP after intravenous administration could be adequately described by a monoexponential equation. Kinetic parameters were calculated using the nonlinear least-squares fitting program, MULTI,¹¹⁾ by a personal computer (PC-9801Vm, NEC). The area under the plasma concentration-time curve (*AUC*) for transdermal data was determined by the trapezoidal rule. The absolute bioavailability was calculated from the ratio of the *AUC* values obtained after percutaneous and intravenous administration.

Statistical analysis was performed using the one-way analysis of variance (ANOVA) with Tukey's multiple comparison procedure. In the case of ointment, differences in each parameter between each formulation were evaluated by the unpaired Student's *t*-test. The significant level adopted was $p < 0.05$.

RESULTS

Pharmacokinetic Studies of DBcAMP Mean plasma levels of DBcAMP after intravenous injection are shown in Fig. 1; the plasma levels declined in a monoexponential manner. Pharmacokinetic parameters calculated using the one-compartment open model are listed in Table 1. The elimination rate constant, k_e $8.24 \pm 1.25 \text{ h}^{-1}$, indicated that the elimination of DBcAMP was very rapid. The biological half-life values of $5.14 \pm 0.81 \text{ min}$ found in our study are in agreement with the value of 8.2 min obtained by Okazaki

Fig. 1. Plasma Concentration of DBcAMP Following Intravenous Administration of 3 mg of the Drug to Rats

Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol.

Table 1. Pharmacokinetic Parameters Obtained from a One-Compartment Open Model after Intravenous Administration of DBcAMP

Parameter	
k_e (h^{-1})	8.24 ± 1.25
$t_{1/2}$ (min)	5.14 ± 0.81
V_d (ml)	42.5 ± 15.3
AUC ($\text{h} \cdot \mu\text{g/ml}$)	9.44 ± 3.18

Dose, 3 mg/head. Each value represents the mean $\pm$ S.D. ($n=4$).

et al.,¹²⁾ following intravenous injection of 10 mg/kg of ¹⁴C-labeled DBcAMP.

Percutaneous Absorption of DBcAMP from Aqueous Solution Mean plasma levels of DBcAMP after the application of an aqueous 3% solution of DBcAMP to rats, and the pharmacokinetic parameters are shown in Fig. 2 and Table 2, respectively. Plasma levels of DBcAMP after its topical application to intact skin were very low, and the systemic availability was $1.2 \pm 0.5\%$, indicating that the percutaneous absorption of DBcAMP from intact skin was negligible. In the full-thickness abrasion model, the plasma levels of DBcAMP rose rapidly and reached a peak within 0.5 h, and elimination of the drug from plasma was very rapid. The systemic availability after the topical application to a site where the skin was excised was $93.1 \pm 34.3\%$. These results indicate that DBcAMP is absorbed very rapidly and almost completely when the aqueous solution is applied in the full-thickness abrasion model. The plasma levels of DBcAMP also rose rapidly after the topical application to stripped skin, but the C_{max} was about one fourth that after application to the full-thickness abrasion model. However, a relatively high systemic availability ($63.9 \pm 14.4\%$) was obtained. The elimination of DBcAMP from plasma was slow compared with the full-thickness abrasion model, probably reflecting the slower absorption.

Percutaneous Absorption of DBcAMP from Ointment Mean plasma levels of DBcAMP after the application of PEG and petrolatum ointment to rats are shown in Figs. 3 and 4, respectively. The pharmacokinetic parameters are shown in Table 3. Plasma levels of DBcAMP after these topical applications to intact skin were very low, indicating only slight absorption had occurred.

Following application of the PEG ointment to a site

Fig. 2. Plasma Concentration of DBcAMP Following Topical Application of DBcAMP Aqueous Solution to Normal Skin (○), Stripped Skin (●) and Full-Thickness Abrasion (▲) Model in Rats

Dose: 30 mg as DBcAMP. Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol.

Table 2. Pharmacokinetic Parameters after the Topical Administration of DBcAMP Solution in Various Skin Models

Formulation	Animal model	AUC (h $\cdot$ μ g/ml)	C_{max} (μ g/ml)	T_{max} (h)	Bioavailability (%)
Aqueous solution	Normal	1.1 ± 0.5^a	0.2 ± 0.1^b	1.3 ± 0.5^d	1.2 ± 0.5^a
	Stripped	60.4 ± 13.6	20.1 ± 9.4^c	0.5 ± 0.0	63.5 ± 14.4
	f.t.a. ^e	87.9 ± 32.4	76.1 ± 38.5	0.5 ± 0.0	93.1 ± 34.3

Dose, 30 mg/head. Each value represents the mean $\pm$ S.D. ($n=4$). *a*) $p < 0.01$ compared to stripped and full-thickness abrasion. *b*) $p < 0.01$ compared to full-thickness abrasion. *c*) $p < 0.05$ compared to full-thickness abrasion. *d*) $p < 0.05$ compared to stripped and full-thickness abrasion. *e*) Full-thickness abrasion.

Fig. 3. Plasma Concentration of DBcAMP Following Topical Application of DBcAMP PEG Ointment to Normal Skin (○), Stripped Skin (●) and Full-Thickness Abrasion (▲) Model in Rats

Dose: 30 mg as DBcAMP. Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol.

where the skin was excised, T_{max} was slower and C_{max} was lower than aqueous solution, but plasma levels were relatively high after 8 h. Following application of the PEG ointment to stripped skin, plasma DBcAMP levels were elevated at 4 h and were relatively high even after 8 h. This indicates that the drug was absorbed gradually. The systemic availability after topical application of the PEG ointment to the stripped skin and the full-thickness abrasion model were 10.6 ± 4.1 and $31.8 \pm 27.4\%$, respectively. Thus, a large part of the drug remained in the ointment 8 h after its application to both damaged skins.

Fig. 4. Plasma Concentration of DBcAMP Following Topical Application of DBcAMP Petrolatum Ointment to Normal Skin (○), Stripped Skin (●) and Full-Thickness Abrasion (▲) Model in Rats

Dose: 30 mg as DBcAMP. Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol.

Table 3. Pharmacokinetic Parameters after the Topical Administration of Various DBcAMP Ointments in Various Skin Models

Formulation	Animal model	AUC (h $\cdot$ μ g/ml)	C_{max} (μ g/ml)	T_{max} (h)	Bioavailability (%)
PEG ointment	Normal	3.0 ± 0.8	0.5 ± 0.1	1.5 ± 1.7	3.2 ± 0.8
	Stripped	10.0 ± 3.9	1.6 ± 0.6	3.0 ± 1.2	10.6 ± 4.1
	f.t.a. ^b	30.0 ± 25.9	8.4 ± 2.2	1.8 ± 1.7	31.8 ± 27.4
Petrolatum ointment	Normal	0.0 ± 0.1^a	0.1 ± 0.2^a	—	0.0 ± 0.1^a
	Stripped	0.6 ± 1.3^a	0.1 ± 0.2^a	—	0.6 ± 1.4^a
	f.t.a. ^b	3.5 ± 0.7	1.4 ± 0.2	0.6 ± 0.3	3.7 ± 0.7

Dose, 30 mg/head. Each value represents the mean $\pm$ S.D. ($n=4$). *a*) $p < 0.01$ compared to PEG ointment. *b*) Full-thickness abrasion.

Following application of the petrolatum ointment to stripped skin, plasma DBcAMP levels were very low. On the other hand, the plasma levels rose rapidly, but C_{max} was relatively low and plasma levels declined rapidly following the application of the petrolatum ointment to full-thickness abrasions. Thus, the plasma concentration profile observed after application of the petrolatum ointment differed from those observed after application of the PEG ointment in the full-thickness abrasion model.

Percutaneous Absorption from Powder Formulation

Mean plasma levels of DBcAMP after percutaneous absorption of DBcAMP bulk powder and various DBcAMP powder formulations in rats are shown in Figs. 5 and 6, respectively. The pharmacokinetic parameters are shown in Table 4. The plasma levels of DBcAMP after topical application of the bulk powder rose rapidly and reached a peak within 0.5 h. The elimination of DBcAMP from plasma was as rapid as had occurred following intravenous injection. The systemic availability and C_{max} after topical application of the bulk powder to stripped skin were higher than after application of the aqueous solution. On the other hand, the plasma levels of DBcAMP after the administration of PW-1, containing only granulated sugar, and PW-3, containing both granulated sugar and PVA, rose gradually. Following the application of PW-2, containing HPC-L, the plasma level was lower than the other two powder preparations.

Fig. 5. Plasma Concentration of DBcAMP Following Topical Application of DBcAMP Bulk Powder to Stripped Skin Model in Rats

Dose: 30 mg as DBcAMP. Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol.

Fig. 6. Plasma Concentration of DBcAMP Following Topical Application of DBcAMP Various Powder Formulation to Stripped Skin Model in Rats

Dose: 30 mg as DBcAMP. Each point represents the mean $\pm$ S.D. ($n=4$). When the S.D. was small, it was included in the symbol. $\circ$, PW-1; $\bullet$, PW-2; $\triangle$, PW-3.

Table 4. Pharmacokinetic Parameters after the Topical Administration of Various DBcAMP Powder Formulations to Stripped Skin

Formulation	AUC (h $\cdot$ μ g/ml)	C_{max} (μ g/ml)	T_{max} (h)	Bioavailability (%)
Bulk	79.6 $\pm$ 17.4	57.7 $\pm$ 21.6	0.5 $\pm$ 0.0	84.3 $\pm$ 18.4
PW-1	12.6 $\pm$ 7.6 ^{a)}	2.6 $\pm$ 1.2 ^{a)}	5.5 $\pm$ 3.0 ^{b)}	13.4 $\pm$ 8.1 ^{a)}
PW-2	2.7 $\pm$ 1.8 ^{a)}	0.6 $\pm$ 0.4 ^{a)}	6.7 $\pm$ 2.3 ^{b)}	2.9 $\pm$ 1.9 ^{a)}
PW-3	12.9 $\pm$ 6.9 ^{a)}	2.5 $\pm$ 1.1 ^{a)}	7.0 $\pm$ 2.0 ^{a)}	13.7 $\pm$ 7.3 ^{a)}

Dose, 30 mg/head. Each value represents the mean $\pm$ S.D. ($n=4$). ^{a)} $p < 0.01$ compared to bulk powder. ^{b)} $p < 0.05$ compared to bulk powder.

DISCUSSION

The results of this study indicated that the percutaneous absorption of DBcAMP from intact skin was negligible in all vehicles examined. This is appropriate for a drug that is to be used for the treatment of ulcers of the skin, and it is considered that evaluation of the formulation of DBcAMP should be done in damaged skin. The degree of ulceration and the condition of diseased skin vary with patients. The subcutaneous tissue is often damaged in a deep ulceration. The bone at the region of ulceration is sometimes partially exposed in a severe case. So we examined two experimental methods for damaging skin. The stripped skin model, which was selected as a model of mildly damaged skin, is the most general and simplest

method of removing the stratum corneum. The full-thickness abrasion model was selected to evaluate the absorption under a severely damaged condition. But only the stripped skin model was used to evaluate the powder formulations because the tendency of the results for these evaluations of ointments appeared to be the same by both methods.

For a topical drug to be effective, it seems better that it not enter the systemic circulation because of its adverse effect, and for such a drug use of the plasma levels to evaluate the formulation is not suitable. However, the results of topical application experiments of aqueous solution and bulk powder indicate that the drug easily penetrates the cutaneous tissue beneath the stratum corneum and rapidly enters the systemic circulation after the drug is released. In addition, none of the additives used in this study are known to influence the diffusion of drug in the skin. Therefore, in the case of DBcAMP the systemic availability is almost equal to the availability at the site of drug action, and plasma level profiles are useful to evaluate the formulation of DBcAMP. Underestimation of AUC is suspected for such plasma profiles as were maintained even after 8 h. However, since DBcAMP injected intravenously or given by application of aqueous solution in the stripped skin or full-thickness abrasion model has a very short half-life, we believe that the difference is insignificant. For this reason, these AUC values are almost equal to the extent of bioavailability up to 8 h.

The absorption of DBcAMP is greatly influenced by the formulation in both damaged skin models. Better absorption was obtained with the PEG than with the petrolatum ointment. DBcAMP was released continuously from the PEG ointment. To decrease the systemic adverse effects of its high levels, it is desirable that the plasma levels rise only gradually. Thus, the PEG ointment has a desirable absorption profile. Yamamoto *et al.* reported that the PEG ointment produced a better clinical effect on decubitus ulcers than the petrolatum ointment.^{4c)} Our results help to explain that result.

Following the application of PW-2, containing HPC-L, the plasma level was lower than the other two powder preparations. These results indicate that the percutaneous absorption of DBcAMP through stripped skin is considerably influenced by the bases used in the powder formulation. The results of topical application experiments of the powder formulations indicate that there is a possibility of developing a powder formulation bioequivalent to the PEG ointment which is used clinically.

The effect of vehicle on percutaneous absorption of active ingredient is complicated.⁵⁾ A drug applied to the skin in various formulations first dissolves and diffuses in the vehicle to reach the vehicle-skin interface, partitions into skin tissue, diffuses within skin tissue, and then enters the systemic circulation.

After the topical application of the bulk powder, DBcAMP is considered to be absorbed rapidly and completely. This would be due to its hydrophilicity, the aqueous solubility of which is more than 500 mg/ml. When bulk powder was administered, DBcAMP was dissolved in exudate rapidly, and higher concentration of DBcAMP

exudate solution resulted in more rapid absorption compared to aqueous solution administration. Since DBcAMP in solution or as bulk powder is rapidly absorbed through damaged skin, differences in rate of absorption by the formulations in this study can be attributed to differences in the rate of drug dissolution and diffusion in the bases or drug release from the bases.

Although the absorption of DBcAMP from petrolatum ointment was very low, plasma concentration profile observed after application of this ointment differed from those observed after application of the PEG ointment in the full-thickness abrasion model. This may be explained by the drug release occurring only from the part of the petrolatum ointment close to the skin. As DBcAMP is insoluble in acetone or chloroform, the dissolution of the drug in petrolatum ointment believed to be very slow.

Concerning the difference of powder formulations in bioavailability, it is not clear whether HPC-L interferes with the release or the diffusion of DBcAMP. In a powder formulation, DBcAMP first dissolves in exudate and releases from the solution of the powder components in the exudate. The bioavailabilities of the powder formulations are influenced by various powder-exudate interactions, such as the exudate absorbing ability of the powder, the diffusion rate of exudate in the powder, or the viscosity of the solution of the powder components in exudate. Especially, the water absorption profile of the formulations can influence their drug absorption in a skin ulcer. Further studies are required to know the effect of the exudate on the bioavailability of a topical drug

formulation in damaged skin.

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