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Evaluation of creams and ointments as suitable formulations for peldesine

Tacey X. Viegas [a,*], Lise L. Van Winkle [a], Paul A. Lehman [b], Sue F. Franz [b], Thomas J. Franz [c]

[a] *BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Birmingham, AL, 35244 USA*
[b] *DermTech International, San Diego, CA, USA*
[c] *University of Arkansas for Medical Sciences, Little-Rock, AR, USA*

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Abstract

In-vitro studies were conducted to study the efficacy of mixed and self-emulsifying creams and hydrophobic ointment formulations in delivering peldesine (BCX-34) into and across cryopreserved human cadaver skin (HCS). Oil-in-water cream formulations, containing 1% w/w of radiolabeled C^{14} BCX-34 and propylene glycol (PG), glycerin (GLY), isopropyl myristate (IPM), oleic acid (OA) and capric-caprylic esters (CE) were prepared. Petrolatum and lanolin based ointments were also prepared with PG. Sections of the HCS, 250 μm thick, were fitted to vertical Franz diffusion chambers containing a receptor medium of pH 7.4 phosphate buffer solution maintained at 37°C. Using the finite dose technique, 4–6 mg of a formulation sample was applied to the epidermal surface of each section and drug diffusion was permitted for 12 and 24 h periods. The distribution of drug into the HCS epidermis, dermis and into the receptor medium was measured by scintillation spectroscopy. The results show good correlation of the calculated in-vitro values for flux and skin-vehicle partition coefficients against the observed amounts of drug detected in the HCS. The mixed emulsion cream formulation containing PG delivered higher amounts of drug into the skin when compared to the same formulation containing GLY cream. The self-emulsifying cream formulation containing IPM had a higher skin-vehicle partition coefficient and delivered more drug into the dermis when compared to those formulations that contained OA and CE. The petrolatum ointment delivered six times more drug into the epidermis than the lanolin ointment, and had higher skin-vehicle partition values. In conclusion, creams containing PG and petrolatum-base formulations would be suitable for BCX-34 dermal delivery.

Keywords: Peldesine; BCX-34; Dermal formulations; In-vitro skin delivery; Purine nucleoside inhibitor; Penetration enhancers

1. Introduction

The enzyme purine nucleoside phosphorylase (PNP) is one of the enzymes in the 'purine salvage

* Corresponding author. Tel.: +1-205-4444600; fax: +1-205-4444640.
E-mail address: tviegas@biocryst.com (T.X. Viegas).

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pathway' that catalyzes the reversible phosphorolytic cleavage of purine ribonucleosides and 2′-deoxyribonucleosides to the purine and the ribose-1-phosphate or 2′-deoxyribose-α-1-phosphate. The pivotal role of this enzyme in T-cell proliferation has been demonstrated in patients with inherited PNP deficiency, where T-cells may be 1–3% of normal. PNP inhibition indirectly suppresses T-cell proliferation and this action may be useful in the treatment and control of various T-cell disorders such as cutaneous T-cell lymphoma, atopic dermatitis and psoriasis (Markert, 1991).

Peldesine (BCX-34) is a small molecule (M.W. 241.25) that was developed as a PNP inhibitor, using structure-based drug design techniques. It inhibits 50% of the PNP at a concentration (IC_{50}) of 36 nM (Bantia et al., 1996). Peldesine (Fig. 1) is a weak base that is soluble in stomach acid and when the pH is < 4.0. It is practically insoluble at neutral pH and will precipitate when the pH is > 5.2. The lipophilicity at this pH when expressed as Log $P_{oct/water}$ is 0.8 (Viegas and Van Winkle, 1999). Preclinical studies in rats and dogs and pilot clinical trials have shown that BCX-34 is stable in biological fluid and tissue and does not undergo metabolism.

A stearic acid based oil-in-water *mixed emulsion* formulation containing 10% by weight of glycerin (similar to cream A below) was selected for earlier trials in healthy volunteers to measure the dermatopharmacokinetics of BCX-34 (Hui et al., 1999). Each cream preparation contained 0.1, 0.3, 1, 3 or 5% w/w of drug and was applied to 1.0 cm^2 sections on the forearm. At a designated time after dosing, each site was washed with soap water and stripped 10 times with cellophane tape and each tape strip was analyzed for drug content. The results show that equilibrium was reached within the first 6 h of cream application. The levels of drug in the upper stratum corneum were significantly higher than the levels in the lower stratum corneum ($P < 0.05$). The need to maximize delivery of BCX-34 into the skin, especially the dermis, led to the reformulation of the BCX-34 dermal preparation. This in-vitro study was performed to:

1. determine whether substituting glycerin (GLY) with propylene glycol (PG) in the formulation would produce significant improvement in drug delivery into and through the skin;
2. evaluate oleic acid (OA), isopropyl myristate (IPM) and capric/caprylic esters (CE) as chemicals that would produce significant improvement in drug delivery;
3. compare the *mixed emulsion* creams, the *self-emulsifying* creams and the *oleaginous* ointments as suitable topical formulations for delivering peldesine into and through the skin;
4. compare the calculated drug flux and skin-vehicle partition ratios with the observed amount of drug measured within the skin epidermis and dermis.

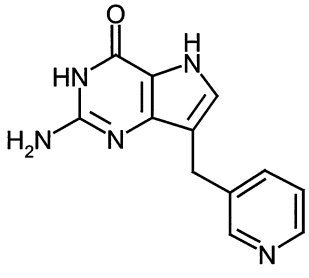

Fig. 1. Structure of peldesine (BCX-34). Solubility of BCX-34 in binary solvent systems (log-linear plot).

2. Experimental

2.1. Materials

Cryopreserved human cadaver skin (HCS) was obtained from a local skin bank in Little Rock, Arkansas. BCX-34 was obtained from the Chemical Development group at BioCryst Pharmaceuticals, Inc., Birmingham (USA) and the radioabeled C^{14}-BCX-34 was purchased from the Southern Research Institute, Birmingham (USA). Glycerin, USP (Optim™) was from the Dow Chemical Company; isopropyl myristate (Emerest 2314) and oleic acid, NF (Emersol 221) were from the Henkel Chemical Group; propylene glycol was from Eastman Chemical Company; capric/caprylic esters (Miglyol 812N and Softisan 378) were from Hüls America; mineral oil, USP (Kay-

dol™) and white petrolatum, USP (White Protopet™) were from Witco Corporation; lanolin alcohol (super Hartolan™) and polyethylene-20-oleyl ether (Volpo-20™) were from Croda Inc.; and all other reagents were compendia and reagent grade.

2.2. Solubility of BCX-34

The saturated solubility of BCX-34 was determined in water, glycerin and propylene glycol and the binary solvent systems of glycerin–water and propylene glycol–water. Excess drug was mixed with known volumes of the solvent mixtures and allowed to equilibrate at 25°C for at least 48 h. The sample mixtures were then filtered through 0.45 μm PVDF filters (Supor LC-13 acrodiscs, Gelman Sciences, Ann Arbor, MI). The filtrates were diluted and assayed by the spectrophotometric method described by Goodin et al., 1995. The saturated solubility of BCX-34 in pH 7.4 phosphate-saline buffer with and without polyethylene-20-oleyl ether (0.5 and 1.0% w/v) was similarly determined.

2.3. Preparation of topical formulations

Cream formulations A and B were triethanolamine-stearate *mixed emulsifier* preparations containing 1% w/w of BCX-34 and either glycerin (GLY) or propylene glycol (PG), respectively. They were prepared as follows: Stearic acid, stearyl alcohol and cetyl alcohol were melted to about 60°C. A blend of radioactive C^{14} and cold BCX-34 (ratio of 1:9) in either glycerin or propylene glycol was added to the molten oily liquid and stirred. Triethanolamine was dissolved in purified water and warmed to 60°C. The water phase was next added to the oil phase with continuous mixing to allow for complete emulsification. The creams were cooled and packaged in glass vials. Samples of cream were assayed for radioactive count (dpm). The pH of these preparations was recorded between 7.8 and 8.0.

Cream formulations C, D, E and F were *self-emulsifying* preparations of 1% w/w of BCX-34 containing propylene glycol alone or with the penetration enhancers isopropyl myristate, oleic acid or capric/caprylic glycerides, respectively (Table 1). They were prepared as follows: Stearic acid, cetostearyl alcohol, emulsifying wax and cetyl ester wax were melted to about 60°C. A blend of radioactive C^{14} and cold BCX-34 (ratio of 1:9) in propylene glycol and a penetration enhancer (isopropyl myristate, oleic acid and capric-caprylic esters) was added to the molten oily liquid and stirred. Purified water was also warmed to 60°C and then added to the oil phase with continuous mixing to permit the oil phase to emulsify with the water. The creams were cooled and packaged in glass vials. Samples of the cream were assayed for radioactive count (dpm). The pH of these preparations was between 6.2 and 6.5.

The ointment formulations G and H with 1% w/w of BCX-34 were prepared by mixing mineral oil, propylene glycol with either lanolin alcohol or petrolatum, respectively. The mixture was heated to 65°C and allowed to melt. A blend of radioactive C^{14} and cold BCX-34 (ratio of 1:9) was triturated into the mixture. The ointments were cooled and packaged in glass vials. Samples of the ointment were assayed for radioactive count (dpm).

2.4. In-vitro human cadaver skin (HCS) distribution

The upright diffusion cell (Franz) apparatus was used in this study. Each jacketed cell had a receptor volume of 4 ml and an opening of 10 mm in diameter (exposed area 0.8 cm^2). The receptor solution (37°C) was made up of a pH 7.3–7.4 phosphated-saline buffer containing 0.5% w/v of surfactant of polyethylene-20-oleyl ether. The selection of this non-ionic surfactant as a wetting agent was first reported by Bronaugh and Stewart, 1986.

Cryopreserved, split-thickness (250 μm) human cadaver skin (HCS) was obtained from a local skin bank and stored in water-impermeable bags at −70°C until used. The skin sections selected were from the posterior trunk section of white Caucasian donors. Prior to use the skin was thawed in 37°C water for 5 min, and cut into sections. A square portion of HCS was placed between the donor and receptor side, epidermal

Table 1
1% w/w BCX-34 formulations and their calculated diffusion parameters following 12 and 24 h of diffusion

Code	Composition of solvents and enhancers	Mean Flux, J_s (ng/cm^2 per hour)		Lag time, τ (h)		Diffusion constant, D (cm^2/h)		Diffusion coefficient, k_p (cm/h)		Partition coefficient, k_m	
		12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
A	10% glycerin	3.29	6.69	1.51	0.61	1.59×10^{-7}	3.92×10^{-7}	3.29×10^{-4}	6.69×10^{-4}	2.48	2.05
B	10% propylene glycol	8.20	13.90	1.63	3.08	1.47×10^{-7}	7.81×10^{-8}	8.20×10^{-4}	1.39×10^{-3}	6.67	21.38
C	7% propylene glycol	2.73	1.98	1.23	1.66	1.95×10^{-7}	1.44×10^{-7}	2.73×10^{-4}	1.98×10^{-4}	1.68	1.65
D	7% propylene glycol + 2% isopropyl myristate	4.63	7.82	1.70	5.28	1.41×10^{-7}	4.55×10^{-8}	4.63×10^{-4}	7.82×10^{-4}	3.93	20.66
E	7% propylene glycol + 2% oleic acid	3.68	2.97	0.66	0.00	3.61×10^{-7}	0.00	3.68×10^{-4}	2.97×10^{-4}	1.22	0.00
F	7% propylene glycol + 7% caprylic/capric glycerides	3.59	2.35	1.47	0.91	1.63×10^{-7}	2.64×10^{-7}	3.59×10^{-4}	2.35×10^{-4}	2.64	1.07
G	7% propylene glycol + 27% lanolin alcohol + 46% mineral oil	2.19	7.57	0.38	1.15	6.40×10^{-7}	2.08×10^{-7}	2.19×10^{-4}	7.57×10^{-4}	0.41	4.37
H	7% propylene glycol + 76% petrolatum + 12% mineral oil	3.09	15.49	2.73	8.04	8.78×10^{-8}	2.99×10^{-8}	3.09×10^{-4}	1.55×10^{-3}	4.22	62.29

side up. The skin integrity of each section was first checked with ^3H_2O by the pulsed finite dose technique described by Franz and Lehman, 1990. The skin sections were considered acceptable if the ^3H_2O flux was less than 1.25 μl/cm^2 and similar to the other sections from the same donor.

BCX-34 absorption was measured using the finite dose technique (Franz, 1978). The diffusion experiments were performed with 4–6 mg cream or ointment samples applied on the HCS epidermis. The drug was allowed to diffuse across the HCS skin over periods of 12 and 24 h. Nine skin sections were used for each formulation and for each diffusion period. At time intervals of 4, 8 and 12 h (12-h study) and 4, 8, 12 and 24 h (24-h study), the receptor solution was removed in its entirety and replaced with fresh solution and an aliquot was analyzed for radioactive content by scintillation spectroscopy. The amount of drug recovered from the receptor medium at each time point was measured. Following the diffusion periods of 12 and 24 h, the skin sections were washed with 70% isopropyl alcohol, removed from the chambers and manually separated into the epidermis and dermis. Each layer was digested in Soluene™ (Packard Instrument Company, Meriden, CT) and an aliquot of the digest analyzed for radioactive count. The amount of drug retained in each skin section was measured.

3. Results and discussion

3.1. Solubility of BCX-34

The saturated solubility of BCX-34 in water, glycerin and propylene glycol is 0.05, 2.15 and 5.09 mg/ml, respectively. The solubility of BCX-34 in the binary mixtures of glycerin–water and propylene glycol–water is illustrated in Fig. 2. The log-linear solubility equation (Rubino et al., 1984) was applied to each curve in order to estimate the solubility power (σ) of each cosolvent.

$$\text{Log } S_m = \text{Log } S_w + f\sigma,$$

Where, S_m is the solubility of BCX-34 in the cosolvent–water mixture, S_w is the solubility of BCX-34 in water, f is the volume fraction of the cosolvent and σ is the solubility power of the cosolvent which corresponds with the slope of the Log S_m versus f curve. The σ values for glycerin and propylene glycol are 1.59 and 2.43, respectively. These values suggest that propylene glycol may be a better solvent than glycerin for BCX-34 in the cream and ointment formulations.

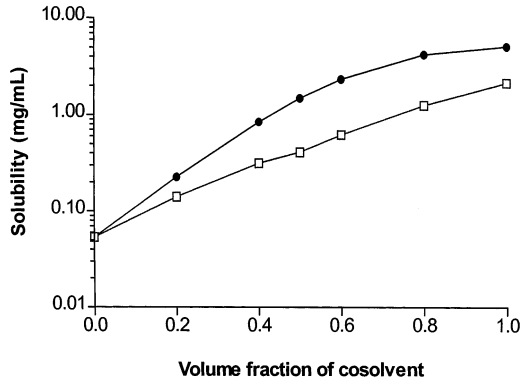

Fig. 2. Key: (□) glycerin and (●) propylene glycol.

The solubility of BCX-34 in the pH 7.4 phosphate-saline buffer was 53 μg/ml and the solubility in 0.5 and 1.0% w/v polyethylene-20-oleyl ether in the buffer was approximately 59 and 67 μg/ml, respectively. There was no significant advantage in using the 1.0% w/v surfactant solution as the receptor medium. Based on the amount of drug in the test sample (approximately 40–60 μg), the receptor solution volume (4 ml), the solubility of drug in this receptor solution, and the fact that the receptor phase was replaced at periodic testing intervals, we believe that adequate sink conditions were maintained throughout the diffusion period.

3.2. In-vitro human cadaver skin (HCS) distribution

The cumulative amount of BCX-34 (ng/cm^2) recovered from the receptor media following the 12 and 24 h diffusion experiments was plotted against time (h). The slope of this line is the flux rate J_s (ng/cm^2 per hour) of drug from the formulation and across the 250 μm skin section. These calculated flux rates were fitted to the following diffusion model equations (Flynn and Smith, 1972; Shah, 1996). The calculated percutaneous

parameters for each formulation are listed in Table 1.

$$J_s = \frac{Dk_m C_0}{\delta} = k_p C_0$$

$$D = \frac{\delta^2}{6\tau},$$

where, D is the diffusion constant within the skin (cm^2/h); δ is the thickness (cm) of the human stratum corneum, reported as 12 μm (Rougier et al., 1999); τ is the lag time (h); k_p is the permeability coefficient through the stratum corneum (cm/h); k_m is the skin-vehicle partition coefficient of the drug; and C_0 is the initial concentration of the drug in the test formulation (ng/cm^3). A careful review of the results showed that higher flux rates correlated with higher skin-vehicle partition ratios, since the thickness of the stratum corneum and the initial concentration of drug in the test preparation are constant.

The mean amounts of drug measured in the HCS epidermis and dermis are recorded in Table 2. The student t-test (two tail) was used to statistically compare the drug delivery differences in the formulations tested. When the *mixed* emulsion creams were first compared, we observed that the propylene glycol cream (B) delivered more amount of BCX-34 into and across the HCS than the glycerin cream (A). After 24 h of diffusion, the measured flux rates were 6.69 and 13.90 ng/cm^2 per hour for A and B, resulting in calculated skin-vehicle partition coefficients of 2.05 and 21.38, respectively. These values are in agreement with the observed amounts of BCX-34 in the dermis, which was 81 and 2280 ng/cm^2 for formulations A and B, respectively (significant difference at $P < 0.01$). When the *self-emulsifying* cream preparations were compared, we observed that formulations C, D, E and F showed no significant differences ($P = 0.01$) in drug delivery after 12 and 24 h of contact with the HCS. However, the flux rate of 7.82 ng/cm^2 per hour for formulation D (containing isopropyl myristate) appeared to be two to three times higher when compared to the rates for formulations C, E and F (significant difference at $P < 0.05$). The skin-vehicle partition ratio was 20.66 and this value is in agreement with the observed amount of BCX-34 in the dermis, 810 ng/cm^2. Formulation E (containing oleic acid) had the smallest skin-vehicle partition ratio with modest amounts of drug detected in the epidermis and dermis. When the ointment formulations G and H were compared, we observe that the petrolatum based formulation H delivered approximately six times more drug into the skin sections than the lanolin based formulation G (significant difference at $P < 0.01$). The flux rate after 24 h of diffusion was 15.49 ng/cm^2 per hour. The measured amount of BCX-34 in the epidermis and dermis was 39 681 and 924 ng/cm^2, respectively, providing a good correlation with the skin-vehicle partition ratio of 62.29. In addition, higher amounts of drug were detected in the epidermis and dermis when compared to those values observed for the *mixed* and *self-emulsifying* cream preparations (significant

Table 2
Distribution of BCX-34 in the epidermal and dermal sections of the human cadaver skin following 12 and 24 h of diffusion

Formulationcode	Amount in epidermis ($\pm$SEM) (ng/cm^2)		Amount in dermis ($\pm$SEM) (ng/cm^2)	
	12 h	24 h	12 h	24 h
A	794.41 ± 291.28	802.63 ± 149.47	291.41 ± 109.19	81.81 ± 22.40
B	2077.03 ± 881.64	7055.01 ± 2153.62	463.94 ± 114.17	2280.21 ± 691.69
C	791.04 ± 204.62	1688.06 ± 505.14	210.54 ± 46.57	315.99 ± 101.16
D	1832.86 ± 331.90	3491.64 ± 823.94	354.22 ± 138.49	810.73 ± 239.84
E	1444.62 ± 246.71	1940.18 ± 890.88	299.29 ± 82.81	520.37 ± 317.32
F	1205.82 ± 266.27	1416.67 ± 500.62	113.75 ± 12.84	147.63 ± 29.58
G	6198.99 ± 669.38	5534.86 ± 1073.10	586.06 ± 198.53	767.13 ± 147.36
H	39122.96 ± 5696.33	39681.03 ± 8556.57	1863.53 ± 321.62	924.93 ± 174.34

difference at $P < 0.01$). Comparison of the *mixed emulsion* cream B and the *self-emulsifying* cream C, show that the former delivered higher amounts of drug across and into each skin section, after 24 h of diffusion (significant difference at $P < 0.01$).

4. Conclusions

Propylene glycol was a better solvent of BCX-34 than glycerin. It has a higher solubilization effect (σ) which relates to a higher thermodynamic activity for the drug at the vehicle-skin interface (Flynn, 1993; Lalor et al., 1994 Lalor et al., 1995). The *mixed* emulsion cream with propylene glycol demonstrated better kinetic and thermodynamic activity when compared to the *self-emulsifying* creams. The difference can be attributed to the chemical composition of these oil-in-water systems. The addition of penetration enhancers such as isopropyl myristate, oleic acid and the capric-caprylic esters produced no significant advantage for the *self-emulsifying* systems. Both the *mixed* and *self-emulsifying* types of creams produced equilibrium in less than 12 h, evidenced by similar diffusion coefficient k_p values in the 12 and 24 h data. These observations are similar to the conclusions reached by Hui et al., 1999. Comparison of ointment and cream formulations point out the advantage of both the lanolin alcohol and petrolatum based formulations over all the cream preparations, in delivering BCX-34 into the dermal layers of the skin, where T-cell inhibition is required. The hydrophobic petrolatum based ointment has better occlusive properties than the lanolin alcohol (wool fat derivative) preparation. The latter is known to absorb water from the skin and produce water-in-oil emulsions (Idson and Lazarus, 1986). The extended contact of this *oleaginous* preparation with the cadaver skin provided increased and sustained delivery of BCX-34 as supported by the flux values and the increased skin-vehicle partition coefficient. In summation, the two non-greasy cream formulations (B and D) would be appropriate for whole body application as is required in the treatment of cutaneous T-cell lymphoma. The ointment formulation H would be appropriate for spot patch or plaque application as in the case of psoriasis.

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