International Journal of Pharmaceutics, 70 (1991) 167–172
© 1991 Elsevier Science Publishers B.V. 0378-5173/91/$03.50
ADONIS 037851739100146 8

IJP 02371

Significance of vehicle thickness to skin penetration of Halcinonide

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(Received 27 September 1990)
(Modified version received 3 December 1990)
(Accepted 14 December 1990)

Key words: Topical delivery; Dose application; Dose thickness; Permeability; Epidermal membrane; Corticosteroid; Halcinonide

Summary

In vitro comparisons of the delivery of the topical corticosteroid halcinonide from a 0.1% cream (Halcinonide Cream USP) have been investigated. Finite dose diffusion experiments through human epidermal membranes were carried out to evaluate the effect of application quantity on the rate of epidermal permeation. At 'clinically' low levels of application (i.e. less than 5 mg/cm^2 of cream) a dose-dependent low rate of permeation was observed. With applications of 5 mg/cm^2 and above the rate of permeation appeared constant although the total amount penetrating the membrane was dependent on the dose. These results produced from carefully controlled experiments using the same skin source emphasise the need for accurate in vitro experimentation to mimic in vivo dose application.

Introduction

Historically in in vitro skin penetration studies, compounds or topical vehicles have been applied to epidermal membrane surfaces at levels approaching gram quantities per square centimetre (Franz, 1978). The choice of these 'infinite' dose experiments is to obtain steady-state kinetics to enable flux and diffusion constants to be calculated.

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However, this in vitro 'infinite' dose/steady-state situation does not parallel in vivo formulation application where a finite amount is spread onto the epidermis in a thin layer (Flynn et al., 1985). As well as providing a variable reservoir of the drug at the skin surface, the relative thickness of this vehicle could influence the rate of permeation of the drug. Drug concentration or solubility may change with evaporation of volatile components from the preparation, altering the thermodynamic potential of the diffusant in the vehicle (Tanaka et al., 1985). Furthermore, a relatively thick vehicle application may result in increased skin hydration.

Recent recommendations by the Food and Drug

Administration (FDA) have recognised the importance of application quantity when conducting in vitro penetration studies from topical vehicles, suggesting that 'the dose per unit area should be equivalent to that normally applied in a single application'. They estimated, assuming a formulation density of 1.0, that a topical dosing should not be greater in depth than 50 μm, equivalent to a formulation application of 5 mg/cm^2 of skin (Skelly et al., 1987).

A controlled study was undertaken, to identify the significance of dose loading on the penetration of the topical corticosteroid halcinonide from a marketed cream formulation (Halog, 0.1% Halcinonide Cream USP) utilising topical applications in the range 1–40 mg/cm^2. The results confirm that dose loading does indeed play a major role in the skin penetration rate and total quantity penetrated and emphasises the need for strictly controlled dose loaded experiments.

Materials and Methods

Materials

Halcinonide cream USP (containing 0.1% halcinonide) was supplied by Princeton Pharmaceuticals, Princeton, NJ.

Permeation studies

Fresh caucasian human skin was obtained following surgical amputation of the lower leg. Within 1 h of excision, human epidermal membranes were prepared by lowering the whole skin (epidermis plus dermis) sample into water at 60 °C for 30 s. The epidermis was then gently removed from the dermis and collected onto aluminium foil prior to storage overnight at −20 °C. The following day, once the epidermal membrane had thawed out, six sections (20 cm^2) were cut from a single membrane. Accurately weighed quantities of Halog cream were applied to the surface of the sections

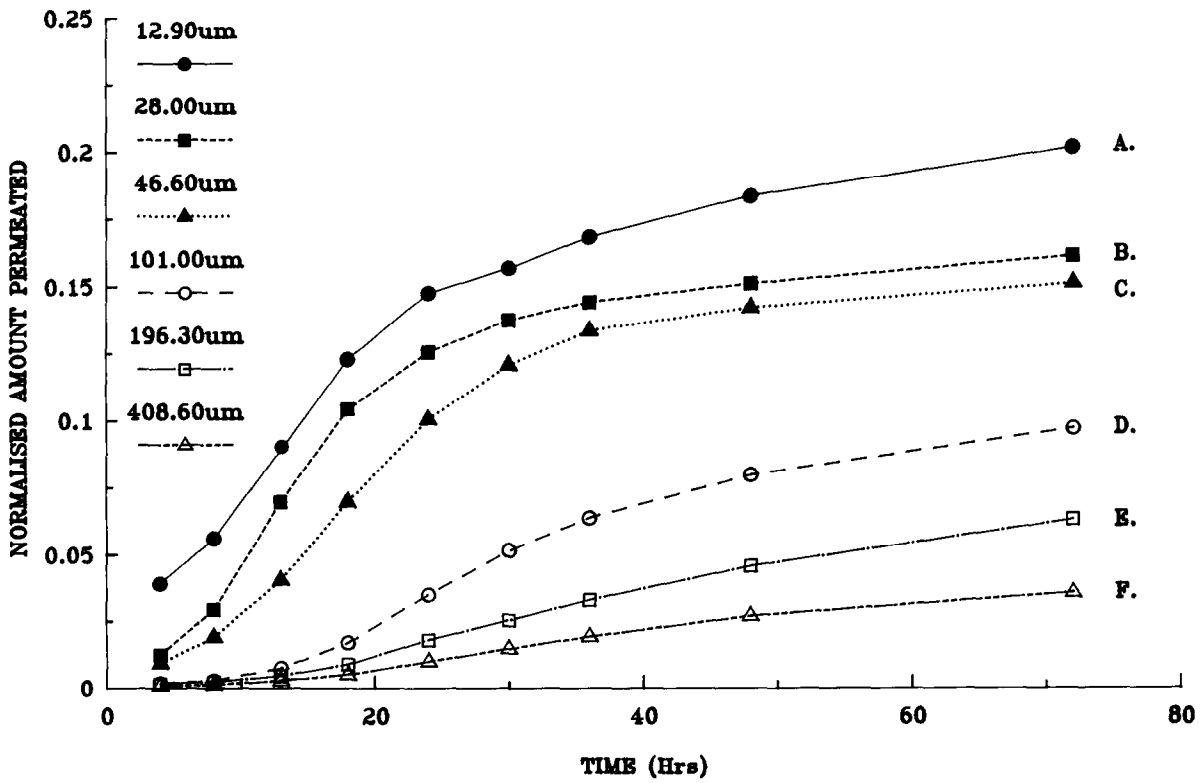

Fig. 1. Topical dose application of Halog Cream (0.1% Halcinonide) to the skin surface: permeation profiles for different skin loadings ($n = 5$).

and spread by means of a small metal spatula. The 'dose loading' applications investigated involved an approximate sequential doubling of the amount from 1 mg/cm^2 through to 40 mg/cm^2.

Individual epidermal membrane discs, diameter 3.2 cm^2, were then cut out from each section, a minimum of four per formulation application. The aluminium foil backing was carefully removed and

TABLE 1

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (1.29 mg / cm^2, n = 5)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	53.61	66.43	133.28	202.13	235.46	249.45	265.75	292.96	314.09
B	126.73	148.28	187.56	216.43	248.20	259.16	274.91	296.77	313.33
C	0.00	0.00	0.00	30.85	63.66	76.12	93.71	107.50	127.64
D	0.00	41.23	73.70	108.44	133.73	142.25	156.73	168.29	206.56
E	73.13	107.06	179.12	235.36	270.65	284.63	294.86	318.72	338.47
Mean	50.69	72.60	114.73	158.64	190.34	202.32	217.19	236.85	260.02
SE	23.91	25.70	35.10	38.71	39.42	39.85	39.13	41.76	40.19

TABLE 2

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (2.80 mg / cm^2, n = 5)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	0.00	41.23	166.06	296.42	379.38	399.45	425.82	455.64	465.54
B	34.12	69.35	190.11	329.27	450.61	538.88	571.02	597.18	628.51
C	0.00	45.82	150.13	235.77	270.38	268.57	282.47	292.24	346.82
D	58.50	114.93	253.80	338.89	359.44	380.22	393.03	411.29	427.84
E	82.86	142.22	214.92	260.58	297.84	336.68	343.96	358.17	388.79
Mean	35.10	82.71	195.00	292.19	351.53	384.76	403.26	422.90	451.50
SE	16.27	19.80	18.34	19.69	31.72	44.61	48.41	51.40	48.47

TABLE 3

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (4.66 mg / cm^2, n = 5)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	0.00	45.82	159.38	329.08	468.08	543.38	591.69	610.66	613.42
B	24.38	63.95	166.22	273.91	340.98	353.51	368.86	379.49	442.76
C	0.00	22.89	118.95	270.10	444.15	559.56	618.10	662.96	651.90
D	155.98	265.31	398.33	528.75	627.62	688.38	611.01	727.83	784.44
E	38.98	51.44	102.86	221.81	460.68	669.14	819.42	926.14	1029.84
Mean	43.87	89.88	189.15	324.73	468.34	562.79	621.82	661.42	704.47
SE	29.00	44.36	53.64	53.76	45.95	59.69	74.80	88.47	97.93

the skin placed dermal side down onto a Franz type, in vitro horizontal glass diffusion cell (Dugard et al., 1984). This methodology attempted to reduce the variations previously noted by ourselves in evenly applying topical vehicles directly to the skin if it was already mounted in a diffusion cell.

The surface area for penetration was 0.64 cm^2 and the receptor volume 1.8 ml. Distilled water was used as the receiving medium, continually stirred with a bar magnet. As the solubility of halcinonide is approx. 10 μg/ml (Kirschbaum, 1979) and the maximum concentration found in the receptor fluid during this study was 0.5 μg/ml,

TABLE 4

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (10.10 mg / cm^2, n = 5)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	0.00	22.89	58.90	139.77	252.69	556.97	702.12	884.43	1101.87
B	0.00	22.89	63.51	155.55	354.13	562.17	707.33	925.83	1196.57
C	34.12	32.71	49.68	96.74	215.07	403.83	594.61	838.13	1061.90
D	19.49	10.83	75.49	196.63	426.06	580.29	671.49	783.72	880.55
E	38.98	58.30	138.99	276.62	420.45	507.21	541.57	590.09	655.60
Mean	18.52	29.52	77.31	173.06	353.68	522.09	643.42	804.44	979.30
SE	8.21	7.99	15.97	30.43	38.02	31.95	32.46	58.59	95.81

TABLE 5

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (19.63 mg / cm^2, n = 5)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	34.12	41.85	96.33	191.03	373.01	567.69	683.42	892.16	1145.04
B	63.37	94.74	142.17	223.94	401.37	564.98	742.16	1017.42	1367.47
C	19.49	31.44	63.85	124.72	229.01	374.10	516.41	762.29	1146.564
D	24.38	36.44	72.45	135.68	229.57	345.28	461.93	697.61	1048.48
E	14.62	31.02	85.50	215.48	445.23	663.83	842.75	1132.27	1480.24
Mean	31.20	47.10	92.06	178.17	335.64	501.18	649.33	900.35	1237.35
SE	8.67	12.07	13.70	20.39	44.92	60.90	70.70	79.91	80.17

TABLE 6

Individual cell accumulated amounts penetrated (ng / cm^2) for topical dose loading (40.86 mg / cm^2, n = 4)

Cell No.	Time (h)								
	4	8	13	18	24	30	36	48	72
A	73.13	100.17	179.93	344.33	580.16	831.18	1054.50	1415.86	1731.39
B	24.38	13.55	64.34	138.74	307.11	490.63	674.42	1008.68	1354.61
C	0.00	18.31	74.83	165.12	378.68	566.76	741.68	1045.99	1408.52
D	48.74	82.03	166.14	201.66	380.13	541.08	689.60	959.13	1326.27
Mean	36.56	53.52	121.31	212.46	411.52	607.47	790.05	1107.42	1455.20
SE	15.73	22.03	30.07	45.81	58.74	76.25	89.32	104.34	93.63

satisfactory sink conditions were provided throughout the study. All the cells were placed in a perspex water bath (Camlab, U.K.) thermostatically maintained at 32°C (±1°C). At predetermined time intervals, 1 ml samples were withdrawn from the receptor compartment by a 1 ml glass Hamilton gas-tight fixed-needle syringe (V.A. Howe, U.K.). Each sample removed was replaced with an equal volume of distilled water maintained at 32°C within the water bath. All samples were then analysed by a sensitive high performance liquid chromatographic method as described by Gardner et al. (1990).

Results

The mean values from the permeation experiments for all the dose loadings are presented in Fig. 1, and individual cell data are shown in Tables 1–6. The results indicate that with increasing amount of cream application there is an apparent similar rise in permeation profile. If quasi-steady state permeation rates are calculated for the respective skin loadings there appears to be a marked increase over the 1–4 mg dose range, levelling out as the dose quantity increases further (see Fig. 2). The 'steady-state' permeation rates were estimated from points 4–24 h for profiles A–C and from 18–50 h for profiles D–F respectively.

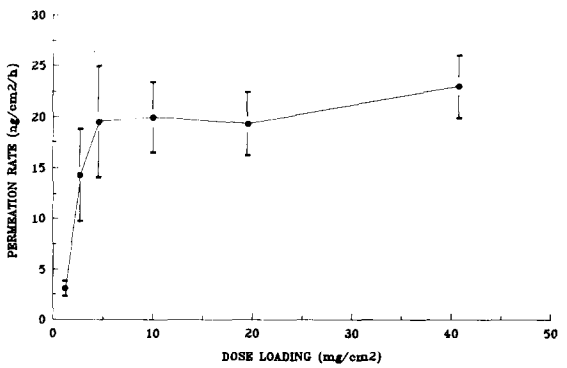

Fig. 2. Graph showing relationship between dose loading and permeation rate.

Discussion

When thin topical applications are applied to the epidermal surface the amount of drug penetrating the stratum corneum, however small, will result in a decreasing drug concentration within the vehicle. This in turn should almost certainly result in non-steady-state kinetics (Addicks et al., 1989). Up to a dose level of 5 mg/cm^2 (profiles A–C, Fig. 1) the accumulated amount starts to plateau out as the concentration of halcinonide at the skin surface is depleted. It is assumed that some of the drug may still be available for penetration within the epidermal layers. Hence, the curves do not completely level out. Above this dosing level (profiles D–F, Fig. 1) the plateau is not so apparent, suggesting that there is an excess of halcinonide available for absorption. However, by 72 h only approx. 20% of the halcinonide would appear to have penetrated. Assuming the majority of the halcinonide is released from the vehicle, it is possible that there is a solubility constraint of halcinonide within the skin lipids. This would appear to be borne out by comparing the relationship between dose loading and the amount penetrated (Fig. 2), where increased dose levels above 5 mg/cm are not mirrored by increases in rates of penetration.

Guy and Hadgraft (1980) have used mathematical expressions to predict theoretical release curves relating the degree of percutaneous penetration to the thickness of the applied base. Assuming a cream density of 1 and a surface area of 1 cm^2, the thickness of our dose loadings fell within the range 10–400 μm. Normalising the accumulated amount by division of the dose applied and relating this to the thickness of the application we have shown very similar curves from our in vitro studies to those predicted for drug release by Guy and Hadgraft (Fig. 3). Deviations in the amount penetrating, being related to the thickness of the applied dose, even at the lower dose levels, i.e. up to 5 mg/cm^2 where the observed amount penetrated over the early time course, up to 13 h post-application, were similar.

From this study we have obtained a good understanding of the effects of dose loading on the in vitro skin kinetics for the topical delivery of the

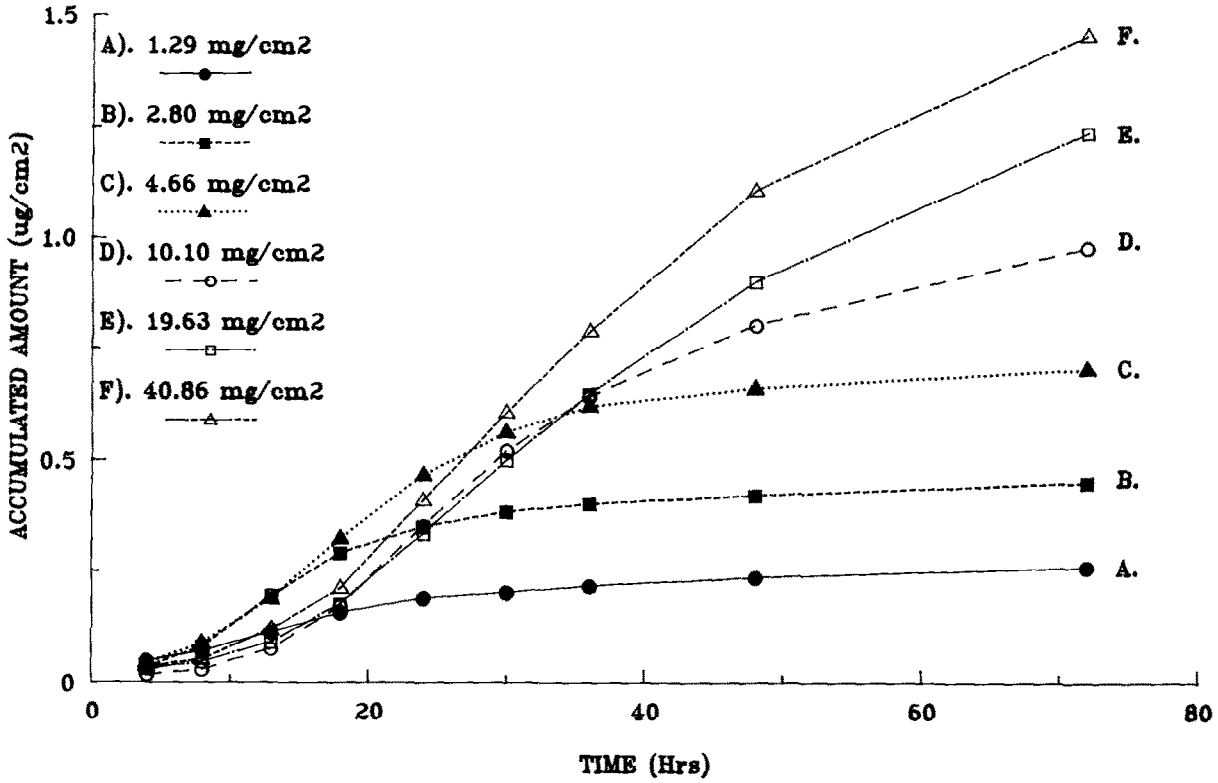

Fig. 3. Profiles for normalised amount penetrated: the effect of varying topical application thickness.

corticosteroid halcinonide. The need to perform accurate, and highly controlled, clinically relevant in vitro finite dose experiments, to mimic the in vivo situation, is emphasised.

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