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A multilayer membrane system for modelling drug penetration into skin *

Reinhard Neubert [1], Christina Bendas [1], Wolfgang Wohlrab [2], Birgit Gienau [1] and Walter Fürst [1]

[1] *Section of Pharmacy and* [2] *Department of Dermatology, Martin-Luther-University, Halle (Germany)*

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Summary

A new model system for modelling drug penetration profiles in human skin is presented with a multilayer membrane system as receptor. For this purpose, membranes were used with dodecanol (DD) as lipid and collodion as matrix. The variability of the model system was demonstrated by the addition of propylene glycol and by changing the DD content of the membrane. Dithranol (DT) was used as a model drug. The penetration of DT was found to take place rapidly into two-, three- and six-layer membrane systems. The penetration profiles of DT in the six-layer membrane systems could be controlled by varying the DD content of the first membrane and by the use of intermediate propylene glycol membranes. Consequently, it was possible to adapt the penetration profiles of DT in the multilayer membrane system to those in human skin.

Introduction

In the development of liberation models, the aqueous receptor phases are replaced by non-aqueous receptor phases (Poulsen et al., 1968; Martin et al., 1989; Niemi et al., 1989; Neubert and Wohlrab, 1990).

Furthermore, the development of absorption or penetration model systems has been focused on the use of two- or three-layer membrane systems for separating topical formulations and receptor phases (Beyer, 1977; Loth and Holla-Benninger, 1978; Winter and Stricker, 1988; Neubert and Wohlrab, 1990). Therefore, a model has been developed with a multilayer membrane system serving as receptor (Fürst et al., 1987; Mank, Neubert and Kawelke, 1989; Neubert et al., 1990). It appears to be possible to simulate the penetration profiles of drugs in human excised skin using this receptor system that can be varied over a wide range.

In this study, the penetration of dithranol (DT) into human excised skin was simulated using a six-layer membrane system. The penetration pro-

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Correspondence: R. Neubert, Sektion Pharmazie der Martin-Luther-Universität, Weinbergweg 15, D-0-4010 Halle/Saale, Germany.

files of DT in the six-layer membrane system have therefore been adapted to those in excised human skin by variation of the lipid content of the membranes applied and by the use of an intermediate hydrophilic membrane. Matrix-stabilized lipid membranes with dodecanol (DD) as lipid and collodion as matrix were used in the model system.

Materials and Methods

Materials

1,2-Propylene glycol (PG), chloroform, methanol, and collodion (4% w/w) were provided by Laborchemie Apolda (Germany). Dodecanol (DD), 2,5-diphenyloxazole and 1,4-di(5'-phenyloxa-2-yl)benzene were provided by United Technologies, Packard (U.S.A.). Dithranol (DT) was purchased from Arzneimittelwerk Dresden (Germany) and vaselinum flavum from Deutsches Hydrierwerk Rodleben (Germany).

Analytical assays

Determination of UV absorbance

The DT content of the membranes was determined by measuring the UV absorbance with the exception of the six-layer systems. For this purpose, the membranes were separated from the multilayer system, removed in a test tube with chloroform, shaken for 30 min, and assayed at 353 nm using a UV-Vis spectrophotometer (VSU 2/P, Carl Zeiss Jena, Germany).

Determination of radioactivity

DT content in the membranes of the six-layer systems was assayed using [^{3}H]DT and a Tri-Carb 300 liquid scintillation counter (Packard, U.S.A.). The membranes were separated from the model system, dissolved in methanol, and mixed with scintillation cocktail (see Wohlrab et al., 1984).

Penetration studies

Multilayer membrane system

The model apparatus consists of polyacrylate (Piacryl®, Piesteritz, Germany) cells. One cell is outlined in Fig. 1. Usually, six cells were fitted together and placed in a chamber maintained at 32 ± 0.2°C during the experimental period. The topical formulation (10 mg) was applied to an exposed membrane area (4.0 cm^2, see Fig. 1), so that the same amount was applied as to excised human skin (Wohlrab, 1984). At selected time intervals (15, 30, 100, 200 and 300 min), the model apparatus was removed from the thermostating chamber, the penetration cells were separated, DT remaining in the formulation was removed, and the membranes assayed for DT content.

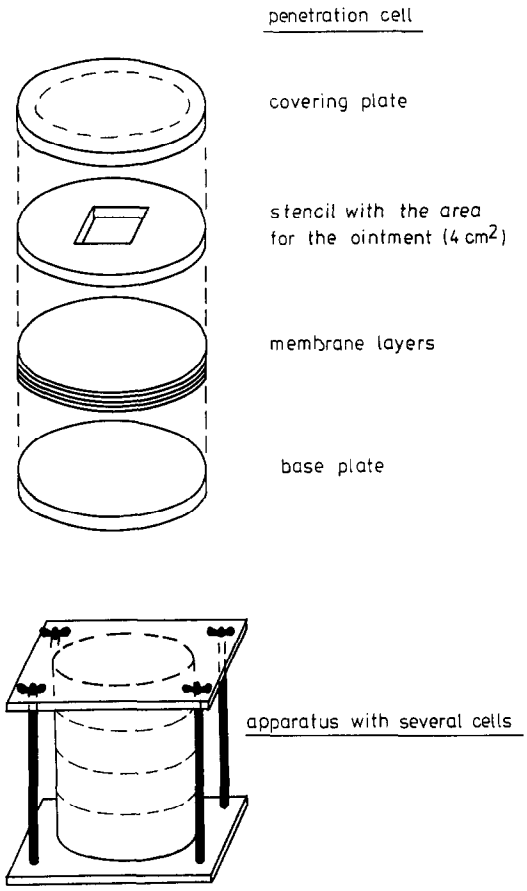

Fig. 1. The model system.

In all experiments DT was incorporated into Vaseline (0.25% w/w) as the model formulation. All experiments were carried out in six-fold.

Preparation of the membranes

The technique for preparation of the membranes has been described previously (Fürst et al., 1980, 1990). The compounds of the membrane were dissolved in a mixture of ethanol and ether (1.5 : 10). This mixture was given on a mercury surface located in a ring of glass of diameter 4.0 cm. This resulted in membranes with a surface area of 3.1 cm^2 being obtained. The petri dishes with the mercury and the glass ring were placed in a desiccator over sulphuric acid. The thicknesses of the DD membranes used are listed in Table 1. The thickness of the membranes was measured using IR spectroscopy as described by Richter et al. (1978). It is reasonable to suppose that the thicknesses of the membranes with PG are not markedly different from those with DD.

Excised human skin

Exposure A 4.0 cm^2 area was marked on the skin section to be tested, which was placed on a synthetic fibre sieve and pinned to the corners without tension. About 16 mg of ^3H-labelled DT vaseline were applied to the test area by means of a spatula and distributed uniformly. The exact quantity of ointment applied was determined from the difference in weight of the spatula before and after application.

Immediately after the application of the ointment, the synthetic fibre sieve was placed in a glass vessel containing a physiologic NaCl solution so that this solution, which was continuously agitated with a magnetic stirrer, was in contact with the lower area of the skin. The whole apparatus was maintained at a temperature of 32 ± 0.2 °C throughout the experiment (see also Wohlrab, 1984; Wohlrab et al., 1984).

TABLE 1

Thickness [a] of the dodecanol (DD) collodion membranes used

DD content of membranes (mg)	Thickness of membranes (μm) ± S.D.
1.0	1.4 ± 0.2
2.0	2.0 ± 0.2
3.7	3.7 ± 0.3
7.5	5.1 ± 1.0
15.0	7.2 ± 0.5

[a] The thickness of the DD membranes was measured using IR spectroscopy as described by Richter et al. (1978).

Penetration measurement

All experiments were performed as double determinations with four different operative preparations each. The procedure on the skin was carried out at 30, 100, and 300 min after application of the ointment. For this purpose, the surface of the skin was first wiped with cotton wool and then fixed to a sublayer of synthetic fibre. Subsequently, a stencil with a 1.0 cm^2 hole was placed on the test area. The horny layer was detached in layers by means of an adhesive film (Tesa® or Prenaband®) and each detachment was transferred separately into a test tube. After removal of the horny layer, several skin cylinders were punched out using a rapidly rotating punch press (diameter 4.0 mm) and horizontal sections excised with a freezing microtome. At first, sections of 10 or 20 μm of a tissue for scintillation counting were each solubilised by incubation with 0.2 ml protosol (New England Nuclear) for approx. 12 h and then treated with 2 ml methanol. The scintillation counting was carried out as described above (see also Wohlrab, 1984; Wohlrab et al., 1984).

Results and Discussion

Single-layer system

Influence of propylene glycol (PG) Firstly, the influence of the addition of PG to the membrane on the penetration of DT into a single membrane was studied. 15 mg of PG were therefore added to a membrane containing 3.7 mg DD. It was found that the addition of PG to a DD membrane increased the penetration of DT (see Fig. 2), so that the modification of the concentration-time profiles could be achieved not solely using PG. However, these results confirm the effects described by Loftsson et al. (1989) that PG is necessary in various topical formulations in order to solubilise lipophilic drugs.

Influence of the DD content of the membranes The influence of the DD content of the membranes used on the penetration of DT was stud-

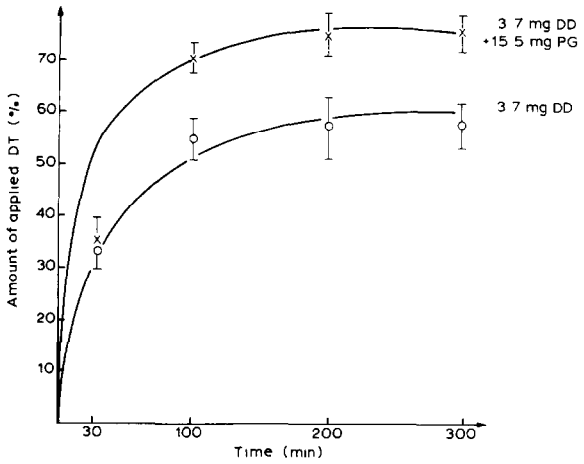

Fig. 2. Influence of the addition of propylene glycol (PG) to the dodecanol (DD) membrane on the penetration of dithranol (DT). Experimental conditions: one-layer membrane; application of 2.5 mg/cm^2 of 0.25% (w/w) DT in vaseline; $X \pm SD$, $N = 6$.

ied in a single-layer system. As shown in Fig. 3, the penetration of DT can be markedly decreased by reducing the DD content of the membranes. Only 8% of the amount of DT applied was taken up by a membrane containing 7.5 mg DD. Above a DD content of 7.5 mg, no further significant enhancement of DT penetration into the membrane was observed (see Fig. 3). Consequently, the adaptation of the penetration profiles seems to be possible by variation of the DD content of the membranes. The relation between the amount

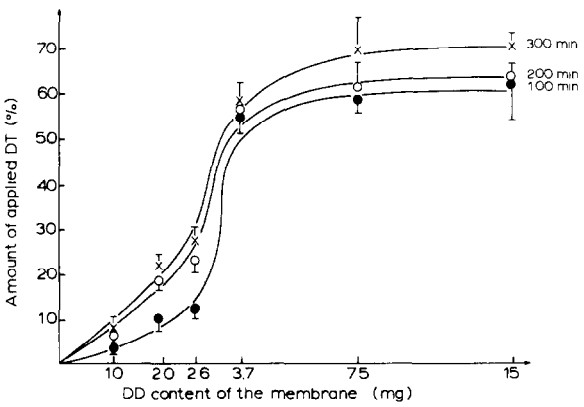

Fig. 3. Influence of dodecanol (DD) content of the membranes on the penetration of dithranol (DT). Experimental conditions: see Fig. 2.

TABLE 2

Penetration of dithranol (DT) from 0.25% (w/w) Vaseline into one-, two- and three-layer membrane systems

Time (min)	Amount of applied DT (%) ± S.D. in			
	One-layer membrane	Two-layer membrane	Three-layer membrane	Sum Σ
100	59.1 ± 2.6			59.9 ± 2.6
200	61.5 ± 6.2			61.5 ± 6.2
300	68.9 ± 7.9			68.9 ± 7.9
100	38.9 ± 0.4	15.6 ± 0.5		54.5 ± 0.5
200	42.5 ± 4.7	23.0 ± 2.9		60.3 ± 7.9
300	33.1 ± 3.7	35.4 ± 2.7		68.5 ± 4.6
100	15.4 ± 1.6	15.1 ± 1.6	18.2 ± 1.4	48.0 ± 0.6
200	33.0 ± 3.2	14.6 ± 3.9	8.6 ± 0.6	54.1 ± 0.7
300	29.8 ± 1.1	23.7 ± 2.4	20.3 ± 1.1	70.9 ± 6.4

of DT taken up by the membrane and the DT content is outlined in Fig. 3. A sigmoidal relation between these parameters was obtained.

Multilayer membrane systems

Comparison of one-, two- and three-layer systems Using membranes containing 7.5 mg DD, it was possible to study the manner in which DT penetrates into multilayer membrane systems. As shown in Table 2, approx. 70% of the applied DT was determined for the uptake after 300 min using a single membrane. However, DT penetrates very rapidly into the second as well as into the third membrane layer, so that the same amount of DT appeared in the receptor system independent of the number of membranes used. It is only after 100 min that a dependence on the number of membranes used appears to exist. In this case, the penetration of DT increased significantly in the sequence three-, two- and one-layer system.

Six-layer systems In order to simulate penetration profiles in excised human skin, six-layer membrane systems are needed to represent layers essential for drug penetration in dermal drug administration (see also Table 4).

Rapid penetration of DT in all membranes was observed in a six-layer system consisting of membranes with an equal DD content (see Table

TABLE 3

Penetration of dithranol (DT) into six layer membrane systems

Expt	Amount of DT in membrane (% w/w ± S.D. of the applied DT)						
	1	2	3	4	5	6	Σ
A	14.7 ±2.2	14.3 ± 3.8	17.4 ±0.4	14.0 ±0.4	12.2 ± 2.7	12.9 ±2.6	85.6 ±2.4
B	20.3 ±0.7 (2.6 [a])	0.63 ± 0.09	0.61 ±0.71	0.52 ± 0.8	0.35 ± 0.5	0.39 ±0.6	22.8 ±2.9
C	20.5 ±4.2 (2.0 [a])	0.67 ± 0.6	0.30 ±0.3	0.12 ± 0.1	0.22 ± 0.27	0.12 ±0.1	21.9 ±4.6
D	7.10 ± 2.1 (1.0 [a])	0.19 ± 0.1	0.11 ±0.05	0.12 ± 0.1	0.10 ± 0.05	0.12 ±0.1	7.73 ± 2.6
E	8.99 ± 2.2 (1.0 [a] + 0.5 [b])	0.81 ± 0.3 (7.5 [a] + 15.0 [b])	0.04 ±0.05 (15.0 [b])	0.03 ± 0.004 (7.5 [a] + 15.00 [b])	0.01 ± 0.001 (7.5 [a] + 15.0 [b])	0.02 ±0.01 (7.5 [a] + 15.0 [b])	8.64 ± 1.1
F	9.13 ± 0.70 (1.0 [a] + 1.0 [b])	0.41 ± 0.28 (7.5 [a] + 3.7 [b])	0.003 ± 0.01 (15.0 [b])	0.03 ± 0.01 (7.5 [a] + 15.0 [b])	0.02 ± 0.01 7.5 [a] + 15.0 [b])	0.03 ±0.02 (7.5 [a] + 15.0 [b])	9.70 ± 0.3
G	8.94 ± 0.64 (1.0 [a])	2.42 ± 2.16 (7.5 [a] + 3.7 [b])	0.02 ±0.01 (15.0 [b])	0.03 ± 0.01 (7.5 [a] + 15.0 [b])	0.01 ± 0.01 (7.5 [a] + 15.0 [b])	0.003 ± 0.001 (7.5 [a] + 15.0 [b])	11.48 ± 1.8

Experimental conditions: see Fig. 1: penetration time, 200 min.
[a] DD content of the membranes (in mg). [b] PG content of the membranes (in mg). All other membranes have 15.0 mg DD.

3, Expt A) as already found in the two- and three-layer systems (see Table 2). On reducing the DD content of the first membrane (see Table 3, Expts B–D) the penetration of DT into these systems was decreased significantly. This effect is not caused by solubility limitations, since the solubility of DT in DD amounts to 2.5 mg/ml at 32 °C and only 25 μg of DT were applied in each experiment. Consequently, it appears to be possible to regulate DT penetration into multilayer membrane systems by varying the DD content of the first membrane. The DD content needed in the first membrane depends upon the DT concentration-time profile in excised human skin (see Table 4). For selecting the DD content required in the first membrane the relationship outlined in Fig. 3 can be used. Only 4.7% of DT applied penetrates into excised human skin.

However, equal amounts of DT appeared in membrane layer 2–6 (see Expt D in Table 3). The significant decrease in the amount of DT in layer 3 of the excised skin in comparison to layer 2 (see Table 4) could not be sufficiently simulated by Expt D in Table 3. Consequently, an intermediate hydrophilic layer seems to be useful in the six-layer membrane system as shown in Expts E–G in Table 3. Hence, a six-layer membrane system consisting of the first membrane with 1.0 mg DD, a third membrane with 15.0 mg PG and

TABLE 4

Comparison of the penetration profiles of dithranol (DT) in excised human skin and in a six-layer membrane system

Layer	Depth of skin (μm)	Excised human skin		Six-layer membrane system [a]
		DT content [b] (%)	AUC (% min)	AUC (% min)
1	10	2.88	589 ±20.8	3439±437
2	20	1.64	323 ± 9.6	951±463
3	40	0.09	13.5± 4.25	201± 96
4	80	0.027	6.3± 0.52	52± 53
5	120	0.041	8.0± 1.30	42± 40
6	160	0.018	5.0± 1.20	20± 23
7	200	0.022	4.7± 0.76	
8	360	0.019	4.7± 0.91	
9	600	0.017	4.1± 0.29	
10	920	0.017	3.6± 0.08	
Sum		4.74	961.9±15.5	4908±516

[a] See Expt G in Table 3.
[b] % of the amount of DT applied after 300 min.

all other membranes with both DD and PG appears to be capable of modelling DT concentration-time profiles in excised human skin. Expt G was carried out as described in Table 3, with samples being removed after 30, 100, and 300 min. The areas under the DT concentration-time curve (AUC) were calculated for the corresponding skin and membrane layer in order to compare DT profiles in excised human skin and in the six-layer membrane system. Previous studies showed that the AUC is more suitable for a quantitative comparison of these penetration systems than the DT content of the membranes and the skin layers in percent (Neubert and Wohlrab, 1990; Neubert et al., 1990). As shown in Table 4, there is a difference between the sum of the AUC values measured in the six-layer membrane system and in excised human skin. On the other hand, differences are also evident between the AUC values of the corresponding single layers. The largest amount of DT is in the first and second layers in both the membrane system and excised human skin. These layers represent the horny layer. The AUC of these layers and the AUC values of the residual layers are greater in the membrane system than in excised human skin. The adaptation of the penetration profiles in the six-layer membrane system used in this study to those in excised human skin is possible to the extent shown in Table 4. Therefore further studies are required in order to determine whether this degree of coincidence in the concentration-time profiles is sufficient for the optimization of topical formulations of DT with the six-layer membrane system.

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