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Evaluation of In-vitro Percutaneous Absorption across Human Skin and in Animal Models

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Abstract—The in-vitro permeability characteristics of human skin have been examined and compared with results in laboratory animals using various types of penetration enhancers. The study was focused on evaluation of predictable validity of the data obtained in animals mostly used in permeation studies. The results in man using the same penetration enhancers were about 30% of the value in the rat. The least potent enhancer was dimethylsulphoxide and the maximum efficacy was observed with sodium laurylsulphate in the rat experiments while in man the results were approximately equal when using any of the studied enhancers. Comparison of the results of experiments performed with *N*-methyl-2-pyrrolidone in several laboratory animals and man showed that the skin permeability in man is approximately 4 times lower than with the rat. Man and guinea-pig were not significantly different in these experiments. There were no significant differences in laurocapram penetration enhancing effect in the concentration range 0·1 to 0·5%, but there was an optimum concentration of laurocapram of 1%. The results showed quantitative differences in percutaneous absorption in various animal species in comparison with man. These differences should be considered in selecting a suitable model for preclinical drug evaluation. The guinea-pig skin penetration seems to be most similar to that in man.

In-vitro experiments in various types of permeation cells to assess absorption through skin are often performed on different animal models (Morimoto et al 1986; Ogiso et al 1986; Ohshima et al 1986; Chien et al 1988; Ritschel & Birkhaus 1988; Tang-Liu et al 1988). However, animal models represent only an experimental arrangement which may yield results widely different from those in human studies. Our experiments were designed to compare the data obtained in an animal model (rat skin) with the results of experiments on human skin, using various penetration enhancers. The selection of a penetration enhancer and its concentration was based on our previous results (Příborský et al 1988). To compare the results in various species used in experimental practice we also made a comparative study in several species, including man, using *N*-methyl-2-pyrrolidone as a penetration enhancer.

Materials and Methods

Materials

Dimethylsulphoxide (DMSO), sodium laurylsulphate (LS) and propylene glycol (PG) were purchased from Lachema (Brno, Czechoslovakia); *N*-methyl-2-pyrrolidone (NMP) from Fluka (Buchs, Switzerland); dodecylazacycloheptan-2-one (laurocapram, Azone); dodecyl-L-pyroglutamate (DLP) from Teijin Co. Ltd (Tokyo, Japan); erioglaucine (Brilliant Blue) from G. J. Dříza (Prague, Czechoslovakia). Other chemicals were of standard analytical grade.

Formulations

Brilliant Blue (50 mg mL^{-1}) was dissolved in distilled water and formulated into solutions containing either laurocapram or DLP at concentrations from 0·0 to 2·0%, LS at a concentration of 2·5%, DMSO 5% or NMP 10%. All penetration enhancers were previously dissolved in propylene glycol, which had a final concentration of 40·0% in all experiments. In controls no enhancer was used.

In-vitro procedure

The experiments were performed with abdominal skin of SPF rats Wistar strain (180 ± 15 g), mice C3H strain (30 ± 2 g), guinea-pigs (350 ± 20 g), rabbits Chinchilla race (2500 ± 120 g) and newborn pigs Hampshire male × Landrace female (F_1), (110 ± 105 g). Human abdominal skin was obtained from the Department of Pathological Anatomy at the University Hospital. All human cadavers were of white race, age between 40 and 55 years with normal values in the standard laboratory tests.

The animals were shaved 24 h before the experiment. After cervical dislocation, full thickness abdominal skin was excised and immediately mounted into the diffusion cells. The cells represented a slight modification of the non-jacketed diffusion cell described by Merritt & Cooper (1984). This cell was composed of a donor and receiver compartment divided by the skin sample. The volume of the receiver compartment was 15·0 mL. A 0·9% sodium chloride solution was used as medium in this compartment. The experimental system represented sink conditions. One mL of a tested formulation containing 50·0 mg mL^{-1} of Brilliant Blue was applied on the stratum corneum surface of the donor compartment. The cells were maintained at 37°C and the effective surface for penetration was 1·77 cm^2. The receiver compartment of the cell was equipped with a magnetic stirring bar, constantly stirring at 150 rev min^{-1}. The samples were taken after 4·5 or 5, 9 or 10, 16 and 24 h.

Analytical procedure

Brilliant Blue was estimated directly using spectrophotometry at 630 nm.

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Kinetic calculations and statistical methods

The in-vitro penetration parameters were calculated from the penetration data:

$$J_T = VdC/dt$$

where J_T is flux, V is volume of the receiver compartment and dC/dt is the rate of change of the penetrant's concentration in the receiver part of the cell. This formula is valid under steady state conditions and unidirectional transport as assumed in our experimental arrangement.

The data are presented as the mean or the mean ± s.e.m. of at least six experiments. Statistical analysis was performed using Student's *t*-test.

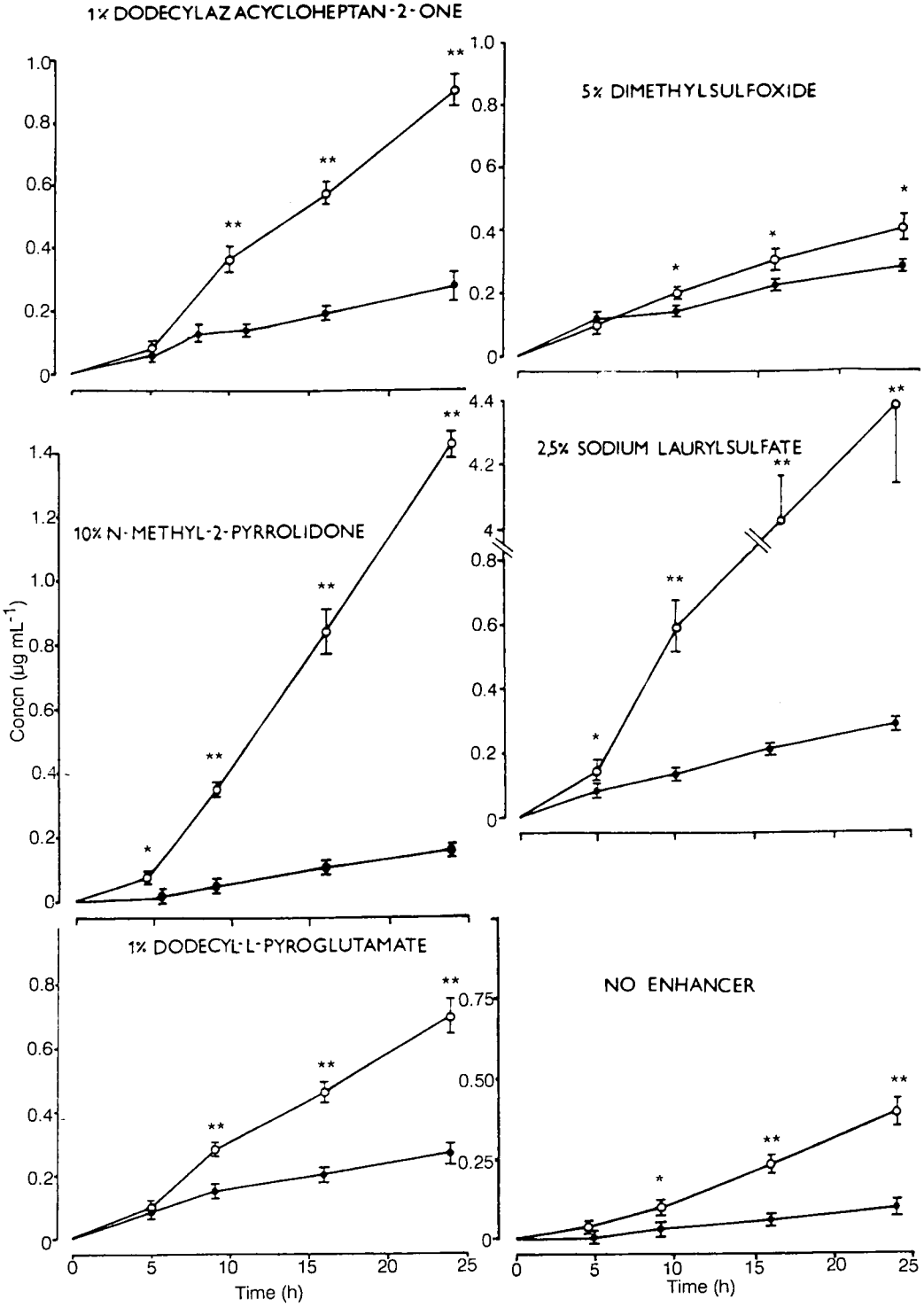

FIG. 1. Mean concentrations of Brilliant Blue ± s.e.m. in the receiver compartment after various penetration enhancers in man ● and in rat ○, n = 6 in each experimental series for both man and rat. Concentration of Brilliant Blue in the donor compartment was 50·0 mg mL^{-1} and propylene glycol 40·0%. (*$P < 0.05$, **$P < 0.01$.)

Table 1. Flux of Brilliant Blue through skin of various species and effect of enhancers.

Enhancer	Species	Flux ($\mu g\ h^{-1}$)	Enhancement
None	Man	0·12±0·019	1·00
	Rat	0·35±0·024*	1·00
Dodecylazacycloheptan-2-one (1%)	Man	0·27±0·020	2·14
	Rat	1·17±0·140*	3·30
Dimethylsulphoxide (5·0%)	Man	0·23±0·018	1·86
	Rat	0·48±0·046*	1·35
Sodium lauryl sulphate (2·25%)	Man	0·30±0·023	2·43
	Rat	6·60±0·520*	18·65
Dodecyl-L-pyroglutamate (1·0%)	Man	0·25±0·019	2·00
	Rat	0·87±0·056*	2·45
N-Methyl-2-pyrrolidone (10%)	Man	0·34±0·021	2·71
	Rat	1·81±0·124*	5·10
	Mouse	2·64±0·440*	
	New-born pig	2·44±0·173*	
	Rabbit	0·57±0·080*	
	Guinea-pig	0·27±0·034	

Concentration of Brilliant Blue (50·0 mg mL^{-1}) in the formulation, propylene glycol 40·0%. Each value represents the mean ± s.e.m. of six calculations. *$P < 0.05$ in comparison with results in man.

Results

Comparison of human and rat skin in in-vitro permeability studies (Fig. 1. Table 1)

Our interest was focused on the comparison of the results from the rat with those obtained in human skin in-vitro using various substances as penetration enhancers. Fig. 1 shows the increase of the indicator concentration (Brilliant Blue). It is evident that in all experiments the concentration increase in man was significantly lower in comparison with the rat.

Comparison of the skin permeability in various species

These experiments were designed to compare several species frequently used in in-vitro skin permeability studies. Ten% NMP was used as a penetration enhancer. Our results showed different permeability in all types of skin as can be seen from Fig. 2 and Table 1.

Study of the optimum effective concentration of laurocapram and DLP in man

The results showed that the optimum concentration of the penetration enhancer significantly improved skin permeability with laurocapram while with DLP no significant differences were observed. The concentrations between 0·1 and 2·0% were used in both enhancers. The results are

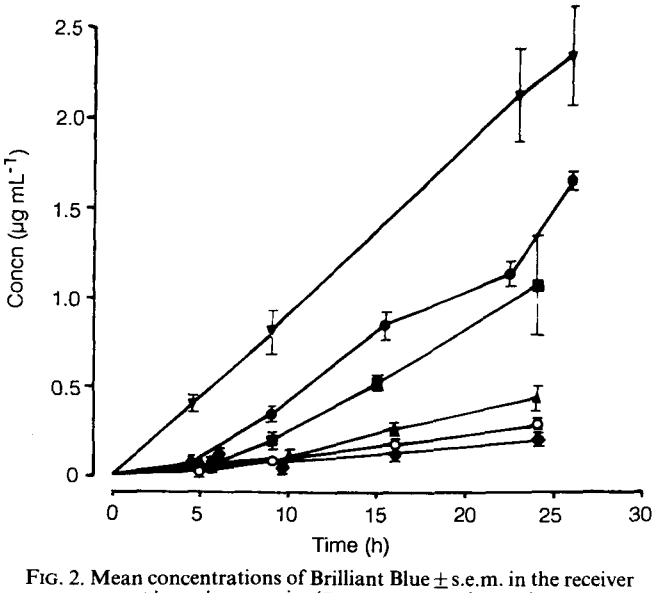

FIG. 2. Mean concentrations of Brilliant Blue ± s.e.m. in the receiver compartment in various species (■ mouse, ▼ newborn pig, ● rat, ▲, rabbit, ○ man, ◆ guinea-pig), n = 6 in each experimental series. Concentration of Brilliant Blue in the donor compartment was 50·0 mg mL^{-1} and propylene glycol 40·0%. 10·0% *N*-methylpyrrolidone was used as a penetration enhancer.

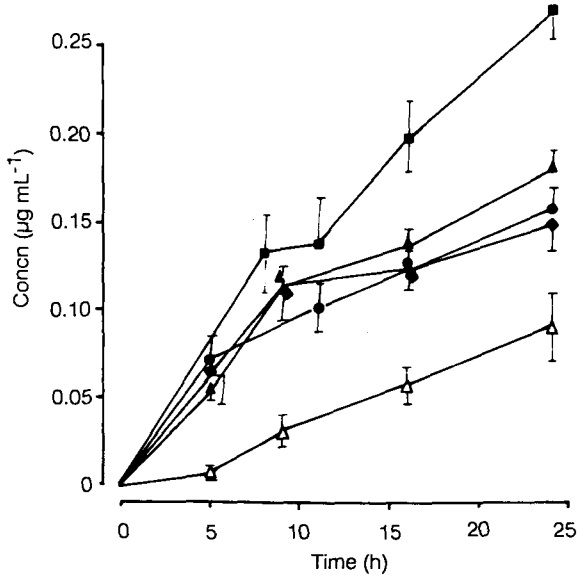

FIG. 3. Human skin. Mean concentrations of Brilliant Blue ± s.e.m. in the receiver compartment after various concentrations of laurocapram (△ no enhancer, ● 0·1%, ◆ 0·5%, ■ 1·0% and ▲ 2·0%), n = 6 in each experimental series. Concentration of Brilliant Blue in the donor compartment was 50·0 mg mL^{-1} and propylene glycol 40·0%.

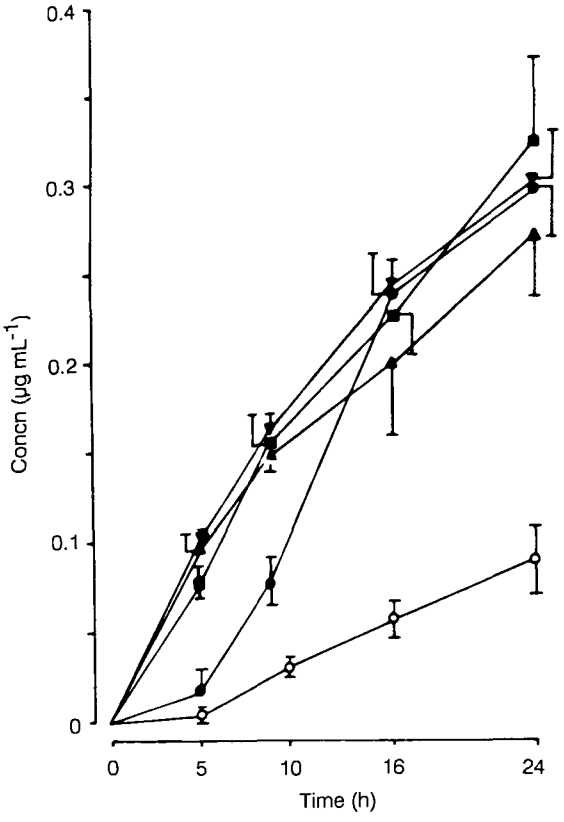

FIG. 4. Human skin. Mean concentrations of Brilliant Blue ± s.e.m. in the receiver compartment after various concentrations of dodecyl-L-pyroglutamate (DLP) (○ no enhancer, ● 0·1%, ■ 0·5%, ▲ 1·0%, ▼ 2·0%), n = 6 in each experimental series. Concentration of Brilliant Blue in the donor compartment was 50·0 mg mL^{-1} and propylene glycol 40·0%.

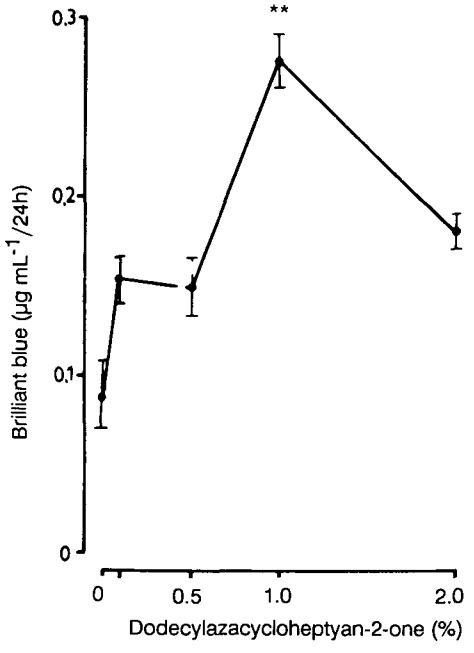

FIG. 5. Human skin. Mean concentrations of Brilliant Blue ± s.e.m. in the receiver compartment after 24 h using various concentrations of dodecylazacycloheptan-2-one, n = 6 in each experimental series. Concentration of Brilliant Blue in the donor compartment was 50·0 mg mL^{-1} and propylene glycol 40·0%. (**$P < 0.01$ comparing maximum concentration with all other values.)

summarized in Figs 3 and 4. A significant improvement of skin permeability was observed when laurocapram 1·0% was used (Fig. 5).

Discussion

Many experiments have been made on animal models to show changes in permeability enhancement by new substances. Few have used human skin (Franz 1975; Aungst et al 1986; Green et al 1988) and comparison among species is also less frequent (Bartek et al 1972). Our main aim was to compare species differences in the skin permeability with special attention to the relationship between man and animal data.

Skin permeability varies not only from species to species but also with age (Guy 1985) and with anatomical site (Schalla & Schaefer 1982). We have tried to eliminate the differences caused by age and anatomical site by using abdominal skin of adult species with the exception of the pig, where newborn animals were used. However, there are still insufficient data concerning skin permeability under pathological conditions of the skin itself or of the organism as a whole. In our experiments we used human skin from subjects with normal basic biochemical parameters since drugs and their formulations can modify pharmacokinetics, e.g. in renal disease (Maher 1984). It is also possible that some diseases may influence percutaneous absorption.

The comparison of the skin permeability of various species (Table 1) shows that the data most similar to those in man were obtained in guinea-pig, while in the more frequently used animals such as rat and mouse the value of the penetration parameter was approximately 5–7 times higher.

The basic data concerning new drug forms are obtained mostly in rats and mice and the results may be confusing for systemic application. This can be seen in the series of experiments using various types of penetration enhancers in which the human skin permeability parameter is consistently lower. The in-vitro interspecies differences shown here are in agreement with the in-vivo interspecies differences reported by Franz (1975) indicating that experiments in-vitro are suitable for screening new drug formulations. The search for optimum concentration of enhancers has shown that the differences in man are less pronounced in comparison with the results in animals when laurocapram and other enhancers are used (Příborský et al 1987). The slight difference in the case of laurocapram and no difference in the case of DLP may be due to the generally lower permeability of human skin.

The aim of our study was to answer some questions dealing with the problems of interpretation of animal data in skin permeability testing. From our results it is clear that good results in animals do not necessarily mean efficacy in man.

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