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Report

Variations of *in Vitro* Nitroglycerine Permeation Through Human Epidermis

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The fluxes of nitroglycerine (NG) through human abdominal epidermis from different individuals were measured *in vitro* at 32°C. The mean NG flux for the entire group was 16.9 μg/cm² × hr, with a standard deviation of 47.7%. There are no significant differences in the means and the variances of NG fluxes between males and females. In groups of different ages (36–58 and >60 years) the mean values are in the same range, whereas the variance is significantly higher in the elderly.

KEY WORDS: nitroglycerine; permeation through skin; variability with age; human epidermis.

INTRODUCTION

In order to test the permeability of drugs through human skin, an *in vitro* model is often used by which the diffusion through human epidermis is tested (1,2). However, human epidermis is not a uniform permeation barrier. It is known that the amount of drug passing through the epidermis varies greatly with the body area from which the epidermis is taken (3,4). Even if the skin is taken from identical body areas of different individuals, wide variations occur, as could be shown for several compounds, e.g., methanol, caffeine, and aspirin (5).

To reduce the number of variables in the screening experiments, skin from an identical body site is commonly used. Nevertheless, the interindividual differences are still high, and therefore, many individuals experiments have to be performed in order to quantify the mean flux of a drug through epidermis.

In the study described in this paper, drug fluxes through the epidermis obtained from the abdomen of several individuals were investigated. The whole collective was divided into different subgroups (males/females, two age groups), and it was evaluated whether significant differences in the mean flux were detectable in the corresponding groups and whether the variability differs among these groups. For this purpose nitroglycerine (NG), which easily penetrates through human epidermis, was chosen as the model compound.

MATERIALS AND METHODS

Chemicals. Nitroglycerine/lactose trituration (1:10) was kindly supplied by Ciba Geigy (Wehr, FRG). Methanol p.a. was obtained from E. Merck (Darmstadt, FRG).

Analytical Instruments. High-power liquid chromatography (HPLC) analyses were performed with a Perkin Elmer LC 601 chromatograph (Perkin Elmer, Überlingen, FRG), a Knauer column, 12.5 × .4 cm (Knauer, Berlin, FRG), filled with LiChrosorb RP 8 (5 μm), a DuPont variable-wavelength ultraviolet (UV) detector (DuPont, Wilmington, Del.) and a Linseis recorder (Linseis, Selb, FRG).

Chromatographic Conditions. The column temperature was ambient. One hundred microliters of the receptor solution was injected. The mobile phase was distilled water/methanol (65:35, v/v); the flow rate, 0.6 ml/min; and detection, UV absorption at 220 nm.

Permeation Studies. Skin permeabilities were measured in glass permeation cells at 32°C (modified from Ref. 6). A piece of epidermis was mounted between two aqueous solution-filled compartments, one containing a drug solution (donor solution) and the other a drug-free solution (receptor solution). The volume of the donor compartment was 11 ml, and that of the receptor compartment was 18 ml. Both compartments were stirred with bar magnetic stirrers at 60 U/min. The area of skin between the compartments was 3.14 cm². The NG concentration in the donor solution was 2.0 mg/ml H_2O. The receptor solution was distilled water. The amounts in the receptor compartment were measured after 1, 2, 3, 4, 7, 12, 24, 36, and 48 hr. "Perfect sink" conditions were maintained during the experiments.

Skin was obtained from the abdomen of 31 pale, Caucasian males and females at autopsy 8–24 hr postmortem (18 females, aged 36–76 years; 13 males, aged 42–76 years). The skin was sealed in evacuated plastic bags, quickly frozen in dry ice, and stored at −20°C, according to the method of Harrison *et al.* (7). Epidermis was prepared according to the method of Scheuplein (8).

Data Analysis. For each sampling point, the flux was calculated as μg/cm² × hr according to Michaels *et al.* (6). For the period when the flux was constant, mean values

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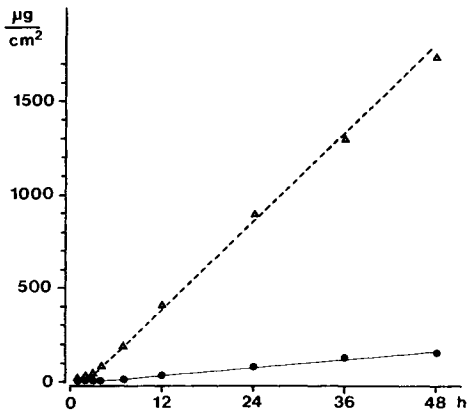

Fig. 1. Time profile of cumulative NG permeation. Most permeable epidermis (△); least permeable epidermis (●).

were calculated for each individual. Mean NG fluxes and standard deviations were calculated for all abdominal skin samples in order to show the interindividual variation of the entire group.

It was also investigated whether there is a correlation between the flux and age, using linear regression analysis. Differences between the male and the female groups and between two age groups (36–58 and above 60 years) were tested for statistical significance using Student's t test and the nonparametric rank sum test of Wilcoxon, Mann, and Whitney. Variances were tested using the F test.

Table I. Nitroglycerine Fluxes Through the Epidermis of Different Individuals

Age (years)	Sex[a]	Flux (µg/cm² × hr)
36	f	19.9
38	f	13.9
42	m	20.5
42	m	23.7
43	m	14.9
49	f	15.3
49	f	16.4
49	m	7.9
50	m	10.9
52	m	19.4
56	f	17.4
57	f	14.0
58	f	19.4
61	m	36.9
61	f	20.4
62	f	15.0
64	m	17.1
66	f	27.8
67	f	9.9
69	m	34.1
70	f	9.2
71	f	19.1
71	m	24.2
71	f	4.4
73	f	4.3
74	f	22.3
75	f	13.6
75	f	28.4
76	f	7.4
76	m	9.6
76	m	6.9

[a] m, male; f, female.

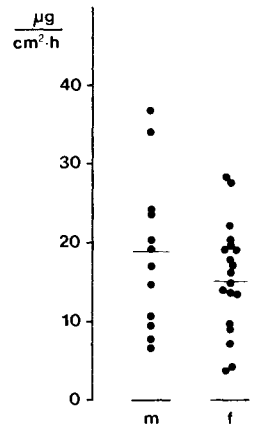

Fig. 2. Comparison of NG fluxes through the epidermis of male (m; N = 13) and female (f; N = 18) individuals. The arithmetic means for each group are also shown.

RESULTS AND DISCUSSION

The cumulative amount of NG per square centimeter passed through the epidermis is plotted against time for the highest and lowest flux in Fig. 1. The fluxes for each of the skin samples are given in Table I. A mean flux of 16.9 µg/cm² × hr was calculated for the entire group, with a standard deviation of 47.7%. The mean flux was 18.86 µg/cm² × hr (±51.7%) in the male group and 15.02 µg/cm² × hr (±42.07%) in the female group. In Fig. 2 the range of variations is shown for the male and the female groups. Statistical differences between the two groups were not detectable (P > 0.2).

NG fluxes could not be correlated with age, either in the whole group or in the male or female group. Investigating different age groups between 36 and 76 years (36–58 and above 60 years), it was found that the arithmetic means were in the same range (Fig. 3). However, the standard deviation in the group above 60 years (58.4%) is clearly higher than in the middle-aged group (26.0%), with the variance in NG

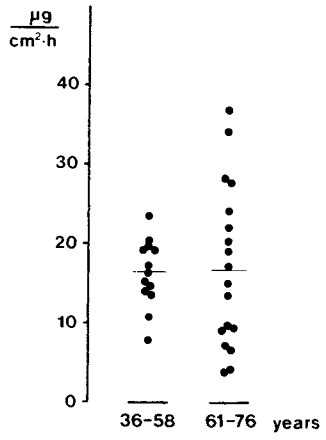

Fig. 3. NG fluxes in different age groups: 36–58 years (N = 13) and 61–76 years (N = 18).

fluxes being significantly higher in the old age group ($P < 0.01$).

In summary, there are no significant differences in the mean fluxes between males and females and between different age groups. We could also demonstrate that large interindividual variations in the amount of NG passing the epidermis exist, especially in the elderly. As the deviations are smaller in the middle-aged, this group should be preferred for screening experiments to define the mean flux through epidermis, which can then be compared to the mean flux of other drugs used in transdermal application forms. Extreme values which heavily influence the mean flux can hardly be found in the middle-aged group.

On the other hand, if the drug turns out to be suitable for further investigation of its transdermal penetration, the flux range is of special interest. Extreme values can occur in the elderly group, i.e., this group may be preferable for the range determinations, especially the minimum flux.

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