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In vivo relationship between transepidermal water loss and percutaneous penetration of some organic compounds in man: effect of anatomic site

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Summary. The relationship between the percutaneous penetration of four chemicals and transepidermal water loss (TEWL) was investigated in vivo in man as a function of anatomic site. The findings showed an appreciable difference in the permeability of the skin from one site to another with regard to both water loss and chemical penetration. In addition, independent of the physicochemical properties of the molecules administered, there was a linear relationship between TEWL and penetration. These data confirm both the importance of anatomic site in the degree of permeability of the cutaneous barrier and the utility of determinations of TEWL and percutaneous absorption in the evaluation of its functional condition.

Key words: Transepidermal water loss – Percutaneous absorption – Anatomic site

Introduction

Barrier function is doubtless one of the most important skin functions. The stratum corneum restricts entry by obstructing the penetration of substances [19, 21] and is involved in the homeostasis of the body, in particular by limiting the loss of water [4, 17, 30], transport mechanisms being diffusional [21, 28]. To date, the relationship between these two aspects of the cutaneous barrier have aroused little investigative interest.

In vivo and in vitro studies performed in man [11] and in animals after UV irradiation [16] have demonstrated that when transepidermal water loss (TEWL) increases, percutaneous penetration increases. However, these studies are qualitative observations and the mechanism linking these two parameters is still unknown.

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Apart from pathologic considerations, the functional state of the cutaneous barrier may vary considerably under physiologic conditions [39]. Thus, in man, cutaneous permeability with regard to applied compounds varies from one site to another [9, 18, 26]. The present study investigates the influence of anatomic site in man in relation to both TEWL and percutaneous absorption in order to establish the precise relationship between these two indicators of the functional state of the cutaneous barrier.

Material and methods

Percutaneous absorption

The percutaneous absorption of four radiolabelled compounds (New England Nuclear): acetylsalicylic acid (carboxyl-^{14}C), benzoic acid (ring-^{14}C), caffeine (1-methyl-^{14}C), and benzoic acid sodium salt (ring-^{14}C) was determined for four anatomic sites, the exact locations of which are shown in Fig. 1. For each molecule and each site, 6 to 8 male caucasian volunteers aged 28 ± 2 years were studied.

Application conditions

In 20 µl of the appropriate vehicle, 1,000 nmol of each compound, with a specific activity adjusted to 10^{-3} µCi/nmol, was applied to an area 1 cm^2. The composition of these vehicles, shown in Table 1, was selected according to the solubility of each compound. Triton X100 was added as a surfactant to obtain smooth spreading of the vehicle over the treated area, the boundaries of which were circumscribed by an open circular cell fixed by silicone glue to prevent any chemical loss.

After 30 min, excess substance was rapidly removed by washing twice (2 × 300 µl) with an ethanol-water mixture (95:5), followed by two rinses (2 × 300 µl) with distilled water and gentle drying with cotton-wool buds.

Measurement conditions

The molecules tested were selected on the basis of the rapidity and high level of their urinary excretion. In view of the literature concerning urinary excretion kinetics for these substances after administration via various routes in different species [5, 6, 8, 10, 24, 25], the total amounts which had penetrated during the 4

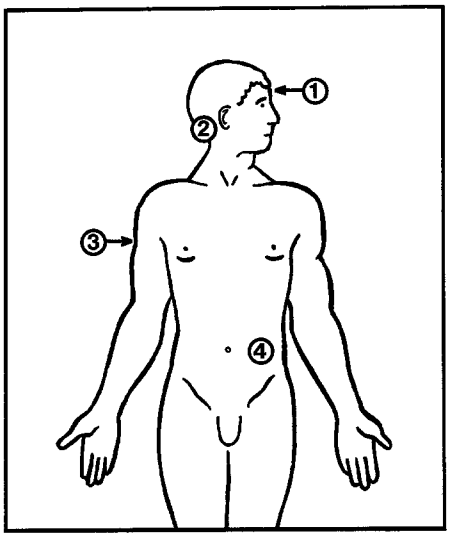

Fig. 1. Anatomic sites tested: *1*, Forehead; *2*, postauricular; *3*, arm (upper, outer); *4*, abdomen

days following application could be calculated, after scintillation counting, from the quantities found in the urines up to 24 h. The proportions of the total amounts of benzoic acid sodium salt, caffeine, benzoic acid, and acetylsalicylic acid excreted within 24 h were 75%, 50%, 75%, and 31%, respectively.

Transepidermal water loss (TEWL)

After topical administration of the tested compound, TEWL was measured with an evaporimeter EPIC (Servo Med, Sweden) from a contralateral site (same anatomic region) in each subject.

The hand-held probe was fitted with a 1-cm tail cimmey extension to reduce air turbulence around the hydrosensors and the metallic shield (supplied by Servo Med) eliminated the possibility of sensor contamination. Measurements ($g \cdot m^{-2} \cdot h^{-1}$) stabilized within 30–45 s. Since the room environment was comfortable ($20 \pm 1°C$) and the subjects physically inactive, the TEWL should closely reflect stratum corneum water flux without gross interference from sweating.

Results

The amounts present in the 24-h urine sample and the total amounts which had penetrated over a 4-day period for each of the anatomic sites investigated (see Fig. 1) are in Table 1. This table also reports the TEWL values obtained on each site tested. Figure 2 presents the relationship between TEWL and total percutaneous absorption of each of the molecules studied for each individual separately but combining the findings for all the sites.

Discussion

The results show (Table 1) that the percutaneous penetration of the test molecules varied with the anatomic location. The areas behind the ear and the forehead

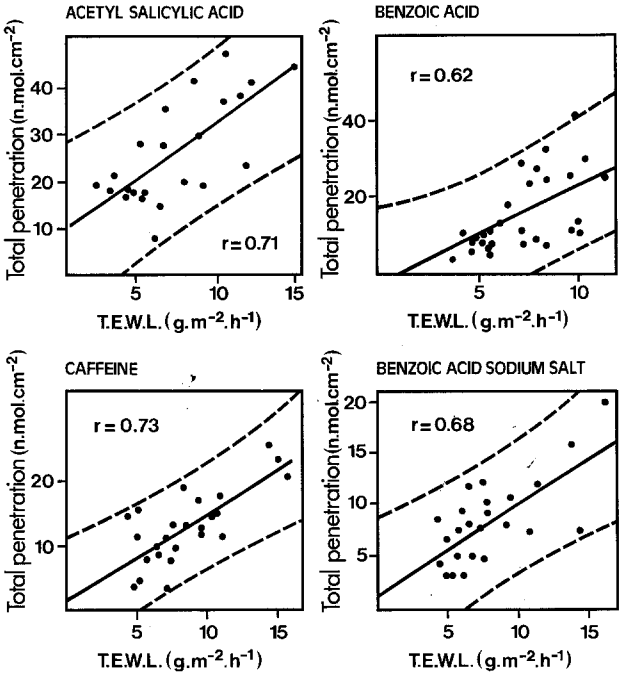

Fig. 2. In vivo relationship between transepidermal water loss (TEWL) and percutaneous absorption of different compounds according to the anatomic site in man

were the most permeable, regardless of the physicochemical properties of the compound tested.

In general, the order of cutaneous permeability was as follows: arm $\leqslant$ abdomen $<$ postauricular $<$ forehead. Although the literature provides few details about the influence of anatomic site on the absorption of molecules, the rank order obtained here agrees with studies performed with other chemicals [9, 10, 18, 35]. Unfortunately, reviews dealing with percutaneous absorption [1, 13, 29] frequently deduce contradictory explanations for the variations in permeability from one anatomic site to another.

Mathematical expressions of the laws of diffusion emphasize the thickness of the membrane crossed. In our opinion, general laws of this type can provide only a theoretical approach to the problem; experience has revealed their deficiencies when applied to discontinuous membranes with the physicochemical complexity of the stratum corneum. Thus, our results show that skin permeability is about two to three times higher on the forehead than on the forearm or the abdomen for both water (TEWL) and molecules (percutaneous absorption, although the thickness and number of cell layers of the stratum corneum of these sites are similar (on average 15 μm and 18 cell layers) [12, 22]. Therefore, the observed differences in permeability on the sites that we have studied have to be explained by additional criteria, such as structural and physico-

Table. 1. Percutaneous absorption and transepidermal water loss (TEWL) values according to anatomic site

n	Anatomic site	Amount in urine after 24 h[a]	Total amount penetrated within 4 days[b]	Transepidermal water loss[c]	Relative permeability to arm	
					Penetration	TEWL
Compound: benzoic acid sodium salt, vehicle A						
6	Arm (upper, outer)	3.02[d] (0.34)[e]	4.02 (0.45)	6.06 (0.36)	1	1
6	Abdomen	5.73 (0.54)	7.65 (0.72)	5.37 (0.46)	1.9	0.9
6	Postauricular	7.54 (0.62)	10.06 (0.82)	7.72 (0.64)	2.5	1.3
8	Forehead	9.31 (1.76)	12.32 (2.30)	12.29 (0.96)	3.1	2
[b] Calculated from urinary excretion: (b) = (a)/0.75						
Compound: caffeine, vehicle B						
7	Arm (upper, outer)	6.04 (0.92)	12.09 (1.84)	7.04 (0.95)	1	1
6	Abdomen	3.76 (0.67)	7.53 (1.34)	6.05 (0.43)	0.6	0.9
7	Postauricular	5.87 (0.52)	11.72 (1.05)	8.74 (0.62)	1	1.2
6	Forehead	11.17 (1.20)	22.35 (2.39)	12.77 (1.05)	1.9	1.8
[b] Calculated from urinary excretion: (b) = (a)/0.5						
Compound: benzoic acid, vehicle A						
8	Arm (upper, outer)	6.87 (0.75)	9.15 (1.01)	4.24 (0.35)	1	1
7	Abdomen	10.88 (1.23)	14.52 (1.64)	4.40 (0.51)	1.6	1
8	Postauricular	16.87 (3.85)	22.49 (5.14)	8.35 (0.41)	2.5	1.9
7	Forehead	20.10 (2.39)	26.80 (3.19)	10.34 (0.70)	2.9	2.4
[b] Calculated from urinary excretion: (b) = (a)/0.75						
Compound: acetylsalicylic acid, vehicle A						
7	Arm (upper, outer)	5.27 (0.18)	17.00 (0.37)	5.08 (0.79)	1	1
6	Abdomen	5.34 (1.03)	17.20 (3.35)	5.16 (0.43)	1	1
6	Postauricular	11.04 (2.50)	29.17 (5.37)	9.04 (0.84)	2.1	1.8
6	Forehead	10.89 (1.02)	35.14 (3.29)	11.22 (0.96)	2.1	2.2
[b] Calculated from urinary excretion: (b) = (a)/0.31						

[c] Measured just before the application, expressed in $g \cdot m^{-2} \cdot h^{-1}$
[d] Expressed in $nmol \cdot cm^{-2}$
[e] SD
Vehicle A: (ethyleneglycol/triton X100) (90/10); Vehicle B: [(ethyleneglycol/triton X100) (90/10)]/(H_2O)(50/50)

chemical properties of the horny layer, whose participation may be relevant to the apparent discrepancies between our present data and previous findings. For, example, no differences in the absorption of hydrocortisone [9], parathion and malathion [18] were found between palms and soles and other sites such as forearm and abdomen, where the horny layer is about 40 to 50 times thinner. On the other hand, it is well established that TEWL on these sites varies greatly. It is about 20–40 g/m^2 per h on palms and soles, while being 4–7 g/m^2 per h for arm and abdomen. These data apparently do not fit with the linear-like relationship between TEWL and percutaneous absorption that we have found in the work reported here (Fig. 2). It is nevertheless possible that, apart from stratum corneum thickness, various morphologic differences of the skin of these sites may partly explain such a contradiction.

For example, the human palm and sole are anatomic sites where the highest distribution of sweat glands can be found. However, at the present time there is no evidence that sweat glands contribute in enhancing or hindering percutaneous absorption of chemicals. On the contrary, it is evident that the water loss on palms and soles is largely overestimated, even in nonsweating subjects, when determined at room

temperature. According to the work of Thiele and Realy [36], sweat glands have two modes of functioning; first, the well-known one, which responds to stress, and, second, a continuous one, where the secretory duct acts as a "heat pipe" which is a high-efficiency calorie exchanger. It can be assumed that at the upper part of the duct, where water vapor condenses before running down the duct walls, some water vapor may escape and thus be taken into account in TEWL. It is worth noting that the second mechanism should be the more important one since it would constantly control the normal thermoregulation. Palms and soles, therefore, may be considered as completely distinct from other skin sites.

From a theoretical viewpoint, it is relatively easy to imagine a molecule's penetration by simultaneously adopting the follicular, sweat, and transcorneal routes. It is unfortunately difficult to evaluate the relative extent of each route. It is conceivable that the higher penetration of areas such as the forehead, where there are more sebaceous glands, might be explained by an increase in transfollicular in preference to transepidermal absorption. However, how can one explain the considerable disproportion (a factor of 50 to 100) in the number of sebaceous glands between the abdomen and the forehead [2, 34] and the relatively slight difference (on average, a factor of 2) detected for the penetration levels of these two areas? In addition, of the total surface area of the appendages, sweat and sebaceous glands account for between 0.1% and 1% of the total skin area, depending on the site involved [3]. Thus, the total volume available for the transport of molecules across structures other than the continuous stratum corneum is probably slight, of the order of 0.01% to 0.1% of the total volume of the skin [3]. It would be hasty and hazardous to conclude that the transfollicular route does not exist on the sole basis of these morphologic considerations and our findings. However, in agreement with several investigations [3, 14, 27, 37, 38] it would seem that the importance of this route in percutaneous absorption phenomena may have been overestimated.

By virtue of its density of active sebaceous glands, the forehead is the richest of the sites studied in terms of sebum. This forms an irregular film of a thickness ranging from 0.4 to 4 μm [15]. It is reasonable to question to what extent the physicochemical nature of the molecule applied interacts with this film and to what extent this initial contact influences the absorption. However, although the real influence of this film on percutaneous penetration requires elucidation, it is of note that its removal or its artificial thickening have no effect on TEWL [7, 15].

In adults, the flat surface of the forehead stratum corneum cells is 30% less than that from other sites, such as the arm or the abdomen [20, 23]. There is an inverse relationship between the area of the corneocytes and the TEWL value [20]. Our results (Fig. 2) show a direct correlation between TEWL and penetration. It is possible that the degreee of percutaneous absorption may itself be inversely linked to the size of the corneocytes.

One of the most important of many current applications of the investigation of the cutaneous permeability in function of anatomic site is the selection of areas most suitable for systemic medication via the transdermal route. The scopolamine transdermal drug delivery systems make use of the postauricular area as the administration site [31, 32]. The wisdom of this choice is confirmed by our findings. The permeability of this area with regard to both water and chemicals is similar to that of the forehead (Table 1). From the morphologic standpoint, the postauricular region presents one characteristic which may partially explain its high permeability. According to Taskovich and Shaw [35], the pronounced insertions of the dermal papilla into the epidermis may favor molecular resorption since the capillaries are nearer the skin's surface.

Every anatomic site has, therefore, its particular features and we have only mentioned some of them. These features, some known, others unknown, combine together in varying degrees to produce the different experimental values of TEWL and penetration observed here. Although most authors recognize the importance of anatomic site with regard either to the degree of absorption of molecules or the TEWL, the literature does not include any quantitative data on the relationship which may exist in man between these two parameters.

Cutaneous permeability is generally considered a mirror of the integrity of the horny layer. Even in normal skin, the efficacy of this barrier is not constant. Thus, as shown in Table 1, for a given anatomic site, the permeability varies widely in relation to the nature of the molecule administered, since this is related to the physicochemical interactions which may occur between the molecule, the vehicle, and the stratum corneum.

For the anatomic sites investigated and for the range of TEWL and penetration observed, there was a linear relationship between the permeability of the skin to the outward movement of water and the inward uptake of molecules (Fig. 2). Table 1 shows that the relationship between the mean values of TEWL and the mean values of percutaneous absorption fit with all the compounds tested, caffeine being an apparent exception (decreased penetration on abdomen and postauricular regions as compared to arm; while TEWL showed a slight decrease on the abdomen and

increase in the postauricular region). It is, however, worth noting that the same relation, when expressed with individual values (Fig. 2), shows correlation coefficients between 0.62 and 0.73 ($P = 0.05$), the latter (and better one) corresponding to caffeine.

With the four molecules investigated, a mean increase of 2.7 in percutaneous absorption corresponded to an increase of 3 in the TEWL value. This fact supports the hypothesis that the efficiency of the barrier is dependent of the physicochemical properties of the molecule administered, but its functional state is independent of it. Consequently, as with determinations of TEWL, percutaneous absorption measurement provides a good marker of the cutaneous barrier integrity.

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