In Vivo Relationship Between Horny Layer Reservoir Effect and Percutaneous Absorption in Human and Rat

DIDIER DUPUIS, ANDRE ROUGIER, ROLAND ROGUET, CLAIRE LOTTE, AND GREGOIRE KALOPISSIS

Département de Biologie, Laboratoires de Recherche Fondamentale de l'Oreal, Aulnay sous Bois, France

The in vivo relationship between the reservoir effect of the stratum corneum and the percutaneous absorption was established in both human volunteers and hairless rats. Four doses of [ring-^{14}C]benzoic acid, 125, 250, 500, and 1000 nmol, were applied on 1 cm^2 either on the external side of the human arm or on the back of the rat, for 30 min. In both human and rat, the data on percutaneous absorption after 4 days is directly related to the dose administered. In rat, the permeability to benzoic acid is twice that of the human skin and the reservoir effect of the stratum corneum, measured at the end of application (30 min), is also doubled for this molecule in rat. Taking into account these findings and the observed histologic differences between the treated areas of these two species, the molecular reservoir of the horny layer may well be situated within the interlayer spaces.

For both species, a linear relationship exists between (x), the amount of substance present in the stratum corneum at the end of application (30 min), and (y), the total amount penetrated in 4 days. The relationship, similar to that obtained in an earlier study on rat with 10 molecules, may be written as follows: $y = 1.83 x - 0.52$ ($r = 0.998$, $p < 0.001$). Thus the amount of substance liable to penetrate through human skin within 4 days can be predicted by measuring the amount present in the stratum corneum 30 min after application of the drug.

In an earlier study in the rat [1] using 10 radiolabeled compounds having differing physical and chemical properties, we showed that a relationship existed between the amounts of substance present in the stratum corneum at the end of application (30 min) and the total amounts penetrated in 4 days, thus allowing a predictive approach to the percutaneous absorption of various molecules in this species. We also noted that, for a given molecule, the relationship was independent of the amount applied. It was still to be verified whether the same phenomenon was observed in other species and, most particularly, in humans. The present study aims at establishing the relationship between the reservoir effect of the stratum corneum and the percutaneous absorption of various doses of benzoic acid comparing human and rat.

MATERIALS AND METHODS

Each dose of benzoic acid was applied to a group of 12 female hairless rats, Sprague-Dawley (OFA) 12 weeks old and weighing 240 ± 10 g, and a group of 5–7 male Caucasian patients, 25 ± 0.8 years and weighing 74 ± 1.6 kg.

Conditions of Application

Doses—125, 250, 500, and 1000 nmol—of [ring-^{14}C]benzoic acid supplied by the Radiochemical Centre Amersham (U.K.) with a sp act

of 0.1 μCi/nmol were applied in 20 μl of a vehicle constituted by ethylene glycol to which 10% Triton X-100 was added as surface active agent. Prior to administration of the compound, the animals were anesthetized by a 0.5 ml/kg i.p. injection of γ-butyrolactone. Benzoic acid was applied onto the backs of the rats and to the external side of the arm of the volunteers, on an area delineated by a 1-cm^2 open cell fixed with silicone glue (Tecsil) in order to prevent spreading. After 30 min of application, the excess substance on the treated area was quickly washed twice (2 × 300 μl) with ethanol:water mixture (95:5) followed by 2 rinsings (2 × 300 μl) with distilled water and light drying with cotton wool.

Measurement of the Percutaneous Absorption

At the end of the application time, the 12 animals in each treated group were equally divided. The first group was used to measure the total amounts of benzoic acid penetrated within 4 days. These were calculated as the sum of the amount found in the following fractions: feces and urine, epidermis and dermis of the treated area (stratum corneum being stripped to discard the nonpenetrated benzoic acid), and the whole body. In the second group, a series of 6 strippings (invisible tape "3M") was carried out on the treated area to determine the amounts of benzoic acid present in the reservoir of the stratum corneum at the end of application.

The method used for these determinations has been published [1]. In the human subjects, each group was treated with a different dose of benzoic acid on the external side of the right arm. Considering both the data in the literature on the urinary excretion of benzoic acid administered by several routes in human and in rat [2,3] and our own results on rat, the total percutaneous absorption within 4 days for benzoic acid applied on humans was deduced from the radioactivity recovered in the urine within 48 h after the application. In order to measure the reservoir effect of the stratum corneum, each patient received on the 7th day after the first application (on the right arm), the same dose of benzoic acid on the external side of the left arm. At the end of the application time (30 min), 15 strippings were carried out on the treated area and the radioactivity in each stripping measured as previously described [1].

Electron Microscopy on Treated Areas

A biopsy of the skin of the external side of the arm in the human and of the back of the rat was sampled in order to comparatively study the horny layer of both species.

The 1-mm^3 samples were submitted to a standard double fixation with an hour-long immersion in a 4% glutaraldehyde solution in 0.4 M cacodylate-HCl buffer (pH 7.4) followed by an hour-long postfixation in a 2% osmium tetroxide solution in the same buffer. After dehydration by ethanol and propylene oxide, the samples were included in Epon 812, then cut crosswise by an ultramicrotome LKB. The ultrafine, 60–70-nm thick, sections obtained were laid on grids covered with Formvar film, then successively contrasted with uranyl acetate and lead citrate, and observed with a conventional JEOL 100 C electron microscope.

RESULTS

Table I gives the kinetics for urinary excretion of the various doses of benzoic acid applied to rat and human volunteers. Table II shows, for the rat, the distribution of benzoic acid in the urines, feces, and in the rest of the body; the amount of benzoic acid penetrated 4 days after administration (Fig 1); and the amounts of benzoic acid present in the stratum corneum 30 min after administration. For the human, Table II shows the amount of benzoic acid excreted in the urine within 48 h after the administration; the amount of benzoic acid penetrated within 4 days (Fig 1), extrapolated from the amount excreted

TABLE I. *Kinetics of urinary excretion of benzoic acid after topical administration*

Benzoic acid applied (nmol/cm²)		Urinary excretion (Time in hours)			
		0–24	24–48	48–72	72–96
125	Rat n = 5	2.13[a] (0.44)[b]	0.18 (0.05)	0.13 (0.03)	0.08 (0.001)
	Human n = 5	0.79 (0.09)	0.13 (0.03)	—	—
250	Rat n = 5	4.04 (0.57)	0.73 (0.31)	0.28 (0.12)	0.19 (0.05)
	Human n = 6	1.71 (0.21)	0.13 (0.03)	—	—
500	Rat n = 5	6.85 (0.51)	0.60 (0.17)	0.49 (0.09)	0.29 (0.04)
	Human n = 5	3.39 (1.11)	0.26 (0.09)	—	—
1000	Rat n = 5	13.67 (1.83)	1.24 (0.40)	0.85 (0.30)	0.49 (0.11)
	Human n = 7	6.89 (2.09)	0.22 (0.40)	—	—

[a] Expressed in nmol/cm² application area.
[b] SD.

TABLE II. *Parameters of percutaneous absorption of benzoic acid*

Benzoic acid applied (nmol/cm²)		Total amounts found 4 days after topical administration					Amounts in stratum corneum of treated area 30 min after application
		Urine[a]	Feces	Epidermis + dermis treated area	Animal body	Total penetration in 4 days[b]	
125	Rat n = 5	2.52[c] (0.43)[d]	0.14 (0.03)	0.05 (0.008)	0	2.71 (0.44)	1.69 (0.18)
	Human n = 5	0.92 (0.06)	—	—	—	1.14 (0.07)	0.81 (0.04)
250	Rat n = 5	5.23 (0.92)	0.48 (0.15)	0.15 (0.06)	0.03 (0.03)	5.89 (1.06)	3.88 (0.69)
	Human n = 6	1.84 (0.22)	—	—	—	2.30 (0.28)	1.61 (0.18)
500	Rat n = 5	8.24 (0.72)	1.06 (0.37)	0.22 (0.05)	0.05 (0.04)	9.57 (0.74)	5.47 (0.64)
	Human n = 5	3.65 (1.20)	—	—	—	4.56 (1.49)	2.64 (0.36)
1000	Rat n = 5	16.25 (2.38)	2.03 (0.27)	0.40 (0.13)	0.06 (0.02)	18.75 (2.57)	10.46 (0.98)
	Human n = 7	7.12 (2.10)	—	—	—	8.90 (2.63)	5.01 (1.06)

[a] For human: amounts found in the first 48 h.
[b] For human: corrected values from (a).
[c] Expressed in nmol/cm² application area.
[d] SD.

in the 48-h urines; and the amounts of benzoic acid present in the stratum corneum 30 min after the application.

Fig 2a,b shows the histologic differences observed by electron microscopy in the treated areas of both species. Fig 3 shows, for human and rat, the relationship existing between the amount penetrated in 4 days of the various doses of benzoic acid applied and the corresponding amounts in the stratum corneum reservoir at the end of administration (30 min).

DISCUSSION

Due to its lack of toxicity [4,5], benzoic acid was chosen. Moreover, it is a well-penetrating substance [4,6] nearly totally excreted in the urine as hippuric acid [2,3]. Effectively, as it has been previously shown [1,6], the kinetics of the urinary excretion of benzoic acid is extremely rapid (Table I). Thus, in both rat and human [3], it has been demonstrated that benzoic acid, administered orally was totally recovered as hippuric acid in the 24-h urines. Furthermore, our present findings in rat show that the amounts of benzoic acid found in 48-h urines following topical application, represent about 80% of the total amount penetrated in 4 days (Tables I, II). This recovery is in agreement with that obtained in this species when benzoic acid was injected i.v. [2]. With these data, it is possible to calculate the total amount of benzoic acid that penetrated in human in 4 days by measuring the excretion in the 48-h urine, considering
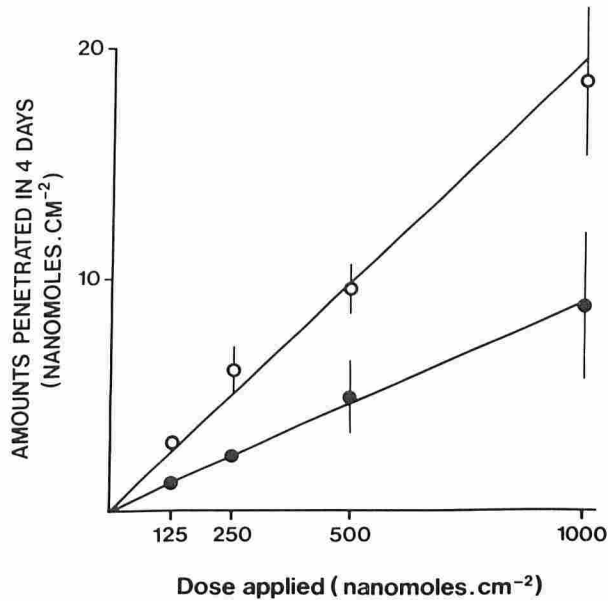
FIG 1. Relationship between dose applied and penetration rate of benzoic acid in rat (O——O) and human (●——●).
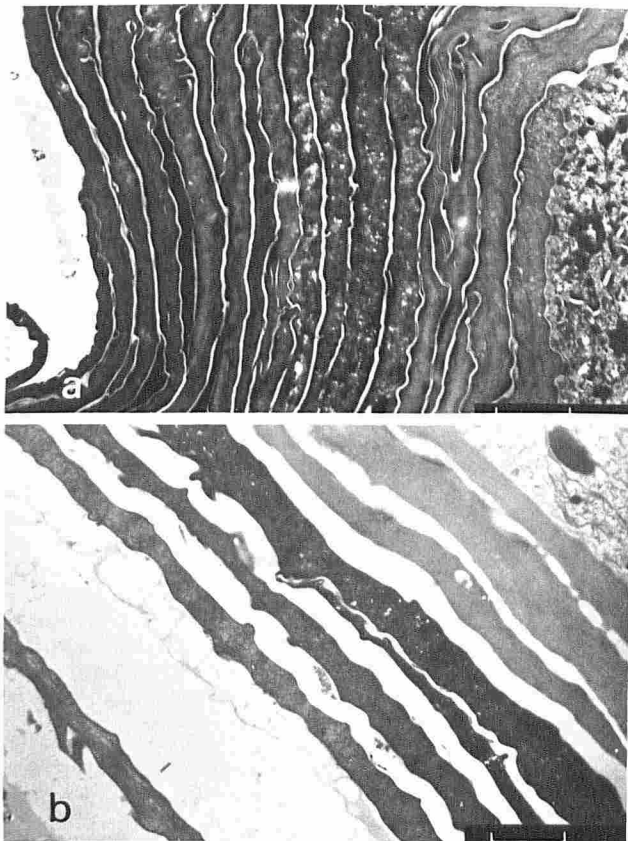

FIG 2. Electron microscopy (transmission): aspects of the stratum corneum of the treated areas in (a) human (external side of the arm) and (b) rat (back). Scale bar = 2 μm.

it represents 80% of the total amount absorbed. The findings on percutaneous absorption of benzoic acid both in human and rat (Table II, Fig 1) show that the amount absorbed is directly proportional to the applied dose. These results are in agreement with those obtained with other substances, in other species [7,8].
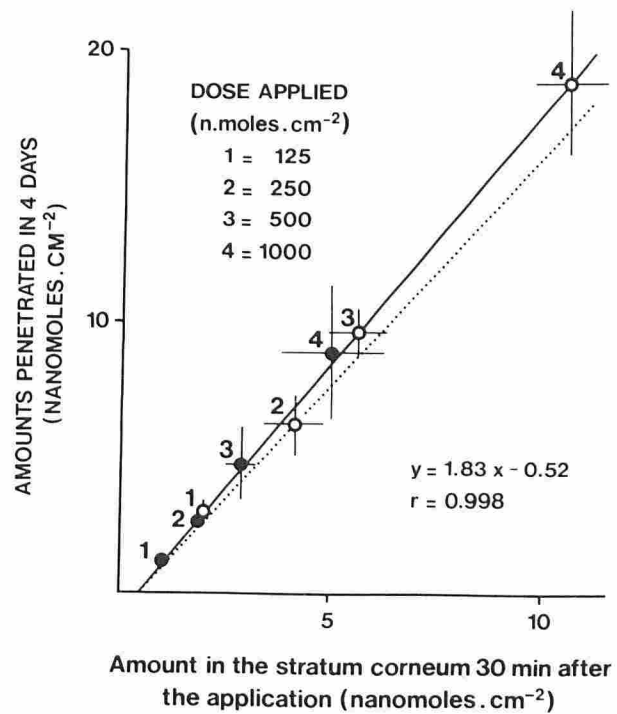

FIG 3. Correlation between the level of penetration of the different doses of benzoic acid after 4 days and their level in the stratum corneum after 30 min of application in rat (O——O), in human (●——●), and in rat with 10 radiolabeled molecules (· · · · ·) [1].

It is common knowledge that percutaneous absorption varies greatly within a species according to the anatomic area selected [9–12]. Bearing this in mind, our results show that the skin of the back of the hairless rat is twice as permeable as the skin of the human arm, whatever the dose of benzoic acid applied (Table II, Fig 1). For each dose of benzoic acid applied, the total amounts present at the end of application (30 min) in the reservoir of the stratum corneum were determined by adding the amounts found on each stripping, both in human and rat (Table II). It may be observed (Fig 3) that, if the stratum corneum reservoir function in rat is twice that of human, the stratum corneum barrier effect is half as much (percutaneous data in rat being twice those in human) (Table II). This statement confirms the directly inverse relationship existing between these two cutaneous functions [1]. It may be admitted that the level of penetration for a molecule is linked to the thickness of the horny layer of the selective anatomic area within a species [11,12]. However, this sole criterion does not suffice to explain the difference existing between species. Indeed, the electron microscopy (Fig 2) showed that in agreement with other authors [13–15], the stratum corneum thickness of the areas studied is nearly similar for both species (about 13–15 μm). Moreover, as can be seen, the number of cellular layers of the human stratum corneum is twice that of the rat (about 16 vs 8), the thickness of each layer being more or less the same in those species (about 0.8–0.9 μm) [16]. The room left for interlayer spaces is therefore much reduced in human (about 0.15 vs 0.7 μm for rat). At this stage of comment, it should be remembered that both the absorption rate of benzoic acid and the reservoir capacity of the stratum corneum for this molecule in rat, are twice those in human (Table II, Fig 3).

It is interesting to speculate whether the differences observed in percutaneous absorption between these two species could be due, not only to the differing number of horny layers, but also to the wide differences in volume of the intercellular spaces, where the molecular reservoir of the stratum corneum could be situated.

Fig 3 shows, for human and rat, and for the various doses of

benzoic acid administered, the linear relationship existing between (x), the amounts present in the stratum corneum reservoir at the end of application (30 min), and (y), the total amount penetrated in 4 days. It is worth noting that the data obtained for both species belong to the same straight line described by $y = a x + b$, where $a = 1.83$ and $b = -0.52$, x and y being expressed in nmol.cm^{-2}. ($r = 0.998$, $p < 0.001$). It should also be mentioned that this curve is nearly similar to that established in a previous study on rat with 10 radiolabeled molecules having very different physicochemical properties [1]. For numerous obvious reasons, it would have been difficult to carry out such a study on humans. Since for rat the correlation curve obtained with 10 molecules is similar to that obtained with increasing doses of benzoic acid, the correlation curve established in human with increasing doses of benzoic acid should be valid for all types of molecules.

In conclusion, these data obtained in human and rat, show that the total amount of a drug susceptible to penetrate in 4 days may be predicted from the amounts present in the stratum corneum 30 min after the application of the drug. Since the linear relationship between these two parameters is the same for human and rat, it could also be a rule for other species.

In addition to being only mildly invasive, this technique should allow studies in human with nonlabeled molecules because of the relatively large amounts of substance present in the stratum corneum at the end of application. It is, however, evident that these results correspond only to a 30-min application time. In which way the duration of application influences the mentioned relationship is a question to which studies are being directed.

REFERENCES

1. Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H: In vivo correlation between stratum corneum reservoir function and percutaneous absorption. J Invest Dermatol 81:275–278, 1983
2. Bronaugh RL, Stewart RF, Congdon ER, Giles AL: Methods for in vitro percutaneous absorption studies. I. Comparison with in vivo results. Toxicol Appl Pharmacol 62:474–480, 1982
3. Bridges, JW, French MR, Smith RL, Williams RT: The fate of benzoic acid in various species. Biochem J 118:47–51, 1970
4. Harvey SC: Antiseptics and disinfectants, fungicides, ectoparasiticides, The Pharmacological Basis of Therapeutics, 6th ed. Edited by A Goodman Gilman, LS Goodman, A Gilman. New York, Macmillan, 1980, p 971
5. Martindale W: In, The Extra Pharmacopoeia, 27th ed, Seventeenth Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series WHO No 539. Edited by A Wade. London, The Pharmaceutical Press, 1979, pp 1275–1276
6. Feldmann RJ, Maibach HI: Absorption of some organic compounds through the skin in man. J Invest Dermatol 54:399–404, 1970
7. Maibach HI, Feldmann RJ: Effect of applied concentration on percutaneous absorption in man (abstr). J Invest Dermatol 52:382, 1969
8. Wester RC, Maibach HI: Relationship of topical dose and percutaneous absorption in rhesus monkey and man. J Invest Dermatol 67:518–520, 1976
9. Marzulli FN: Barrier to skin penetration. J Invest Dermatol 39:387–393, 1962
10. Lindsey O: Percutaneous absorption, Proceedings of the XII International Congress on Dermatology. Edited by DM Pillsbury, CS Livingood. Amsterdam, Excerpta Medica, 1963, pp 407–415
11. Feldmann RJ, Maibach HI: Regional variation in percutaneous penetration of ^{14}C cortisol in man. J Invest Dermatol 48:181–183, 1967
12. Maibach HI, Feldmann RJ, Milby TH, Serat WF: Regional variation in percutaneous penetration in man. Arch Environ Health 23:208–211, 1971
13. Christophers E: Cellular architecture of the stratum corneum. J Invest Dermatol 56:165–169, 1971
14. Holbrook KA, Odland GF: Regional differences in the thickness (cell layers) of the human stratum corneum: an ultrastructural analysis. J Invest Dermatol 62:415–422, 1974
15. Anderson RL, Cassidy JM: Variations in physical dimensions and chemical composition of human stratum corneum. J Invest Dermatol 61:30–32, 1973
16. Marks R, Barton SP: The significance of the size and shape of corneocytes, Stratum Corneum. Edited by R Marks, G Plewig. Berlin/Heidelberg/New York, Springer Verlag, 1983, pp 161–170