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Review Article

RELEVANCE OF ANIMAL MODELS FOR PERCUTANEOUS ABSORPTION

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INTRODUCTION

The ideal way to determine the penetration potential of a compound in man is to do the actual study in man. Mechanisms and parameters of percutaneous absorption elucidated in vivo with human skin are most relevant to the clinical situation. However, many compounds are potentially too toxic to test in vivo in man and so their percutaneous absorption must be done in animals. Likewise, in vivo human studies are costly and not all investigators have access to human volunteers. Mechanism studies and studies on parameters affecting absorption must therefore be explored using animals and in vitro techniques.

The following is a discussion of the validity of animal models for percutaneous absorption. This discussion is preceded by a brief description of the methods used for determining percutaneous absorption, and of some of the parameters which affect absorption. This is necessary because, in many of the comparative studies, factors other than species were varied.

METHODS USED IN PERCUTANEOUS ABSORPTION

The main methods for measuring percutaneous absorption are the in vitro diffusion cell technique and in vivo determination. The in vitro technique involves placing a piece of excised skin in a diffusion chamber, applying compound to one side of the skin, and then assaying for compound in the collection vessel on the other side of the skin (Tregear, 1966). Excised human and animal skin are used, and the skin can be intact or separated into epidermis or dermis. Artificial membranes have also been used in place of skin to measure diffusion kinetics.

Percutaneous absorption in vivo is usually determined by the indirect method of measuring radioactivity in excreta following topical application of the labeled compound. Plasma levels of compound are extremely low following topical application, usually below assay detection level, so it is necessary to use tracer methodology. The labeled compound, usually carbon-14 or tritium, is applied to the skin. The total amount of radioactivity

excreted in urine or urine plus feces is then determined. The amount of radioactivity retained in the body or excreted by some route not assayed (CO_2, sweat) is corrected for by determining the amount of radioactivity excreted following parenteral administration. This final amount of radioactivity is then expressed as the per cent of applied dose which was absorbed (Feldman and Meibach, 1969a and b). Plasma radioactivity can also be measured and the percutaneous absorption determined by the ratio of the areas under the plasma versus time concentrations following topical and intravenous administration (Wester and Noonan, 1978). It should be pointed out that radioactivity in blood and excreta can include both the applied compound and metabolites. Metabolism of compound by skin as it is absorbed will not be detected, nor will it be reflected in the per cent absorption determination. The only way to determine the absolute bioavailability of a topically applied compound is to measure the compound by specific assay in blood or urine following topical and intravenous administration. However, this is extremely difficult to do since plasma concentrations after topical administration are often very low.

The other in vivo method of estimating absorption is to use a biological/pharmacological response. Here, a biological assay is substituted for a chemical assay, and absorption determined. An obvious disadvantage to the use of a biological response is that it is only good for compounds which will elicit an easily measurable response.

PARAMETERS WHICH AFFECT PERCUTANEOUS ABSORPTION

Drug concentration and surface area

When a compound comes in contact with skin the amount of absorption will depend on many parameters. Foremost among these parameters is concentration of applied dose and surface area. As the concentration of applied dose increases, the efficiency of absorption (per cent) can change. However, a more relevant point is that as the applied dose is increased the total amount absorbed into the body increases (Wester and Meibach, 1976; Scheuplein and Ross, 1974). The other parameter closely associated with dose is surface area. Increasing the surface area of applied dose increases the absorption (Noonan and Wester, 1980). Therefore, the greatest potential for percutaneous absorption can occur when a high concentration of compound is spread over a large part of the body.

Skin site of application

Variation in absorption occurs depending on which anatomical site the compound is applied to. This is true for both man (Feldman and Meibach, 1967; Meibach et al., 1971) and animals (Wester et al., 1980a). In man the anatomic site of greatest absorption is the scrotum where most of the applied compound is absorbed (Feldman and Meibach, 1967). Other anatomic sites of high absorption are the scalp, forehead and post-auricular region.

Occlusion

Percutaneous absorption is increased if the site of application is occluded. Occlusion is a covering of the applied dose, either intentionally as with bandaging or unintentionally as putting on clothing after applying a topical compound. A vehicle such as an ointment can also have occlusive properties. Occlusion results in a combination of many physical factors affecting skin and the applied compound. Occlusion changes the hydration and

temperature of the skin and these physical factors affect absorption. Occlusion also prevents the accidental wiping off or evaporation (volatile compound) of the applied compound, in essence maintaining a higher applied dose.

Skin condition

There are skin conditions other than hydration and temperature which will affect percutaneous absorption. The most obvious condition is loss of barrier function of the stratum corneum through disease or damage. Absorption can be virtually 100% if all barrier function is removed. Skin condition also changes with age. The genesis of the stratum corneum occurs during gestation and it probably is concluded by birth (Singer et al., 1971). Preterm infants probably do not have a fully developed stratum corneum and therefore they have increased skin permeability (Nachman and Esterly, 1971). Skin of the elderly also undergoes change and this can influence absorption. Virtually any type of change in skin condition, especially change in the barrier function of the stratum corneum, whether natural or inflicted, will change the percutaneous absorption of the skin.

Vehicle

A.H. Beckett stated that 'a drug is not given to man: what is given is a preparation containing the drug'. This is true for topical vehicles because they influence the percutaneous absorption of the drug and they exert their own effect on the skin. It is not within the scope of this report to discuss the many kinds of vehicles or the interactions between vehicles, skin and drug. Percutaneous absorption of a drug from a vehicle depends on the partition of the drug between the vehicle and the skin, and the solubility of the drug in the vehicle. In addition to drug solubility, factors such as drug concentration and pH can influence the interaction between vehicle, drug and skin. The vehicle can change the integrity of the skin and this will influence absorption. An example would be an occlusive vehicle which would alter skin hydration. Vehicles can contain an agent such as urea which will enhance percutaneous absorption, or the vehicle itself will enhance absorption. The best example of the latter is dimethylsulfoxide (DMSO) which readily permeates skin and by 'solvent drag' causes enhanced penetration. The vehicle is so important that its influence on absorption should not be minimized in an absorption study.

Multiple dose application

Percutaneous absorption studies are usually conducted using a single application. By some analytical means the amount absorbed is determined and the percentage of absorption of that compound is calculated. This is fine for the limits of the study, but the question remains as to its relevance to the clinical situation. A topically administered compound (prescribed or exposed contamination) can be applied more frequently than once per day, and the topical exposure can be on a chronic basis. This is a new area in the pharmacokinetics of skin absorption. Initial studies show a difference in skin absorption with repetitive daily application and with daily chronic application. Absorption from one application of a high concentration (hydrocortisone, testosterone) was greater than when the same concentration was applied in equally divided doses (Wester et al., 1977, 1980a). In another study the absorption of hydrocortisone significantly increased during chronic administration. Absorption on the 8th day of application was 144–273% greater than on

the first day of application (Wester et al., 1980b). Similarly with chronic administration of salicylic acid the penetration flux increased during the first 5 days of application Penetration flux then decreased with weekly application (Robers and Horlock, 1978). In both of these studies involving chronic application the authors suggest that the initial applications of compound altered the condition of the stratum corneum and this altered barrier function resulted in the different absorption for subsequent applications.

Metabolism

One of the methods which the body uses to protect itself is the metabolism of foreign substances and drugs. The organ most associated with metabolism is the liver. Any ingested substance as it is absorbed from the gastrointestinal tract must first pass through the liver. Here any potentially toxic material can be biotransformed into inactive metabolites. Other 'first contact' organs, the lung and skin, are also capable of metabolizing drugs and foreign substances. The skin contains most of the functionalization reactions of liver, the phase I oxidative, reductive and hydrolytic reactions, and the phase II conjugation reactions. The metabolizing potential of skin has been estimated to be about 2% that of the liver (Pannatier et al., 1978).

Since the skin possesses many of the same enzymes as the liver, it would be interesting to compare their relative activities. This would be important if the activity of the skin were sufficient to allow it to serve as an alternate metabolic site for systemically (e.g. i.v. or oral) administered drugs. The activities of several cutaneous enzymes have been measured and compared to hepatic activities (Pohl et al., 1976). The activities of these enzymes in the skin are quite low compared to the liver (typically 2–6% of the hepatic values). Although these data indicate that cutaneous metabolism is very low, this may not be representative of the in vivo situation.

The distribution of skin metabolizing enzymes in skin is an important consideration. Laerum (1969) found that oxygen consumption was 5.4-fold greater in the epidermis than the dermis. Bamshad (1969) found that the enzyme catechol-O-methyl transferase was 8.3-fold greater in the epidermis. Weinstein et al. (1968) found that the epidermal metabolism of estradiol to estrone was much greater than metabolism by the dermis. Finally, Chapman et al. (1977) showed that 96.5% of aryl hydrocarbon hydroxylase activity in the skin was present in the epidermis. Therefore, these data indicate that most of the enzyme activity of the skin may be localized in the epidermal layers.

The epidermal thickness ranges from 0.06 to 0.1 mm. The dermis may be 2–4 mm thick. Therefore, the epidermis makes up only 2.5–3% of the total skin. This percentage may be even smaller if the subcutaneous layers are included in this calculation. The cutaneous enzyme activities reported in the literature were based on enzyme activities in whole skin homogenates. Assuming that these enzymes are constrained to the epidermal layer, the real activities range from 80 to 120% of those in the liver. Therefore, cutaneous enzymes are quite active.

If the enzyme activity of the epidermis is very high, then perhaps the skin may serve as an alternate site for the metabolism of systemically available drugs. The liver is normally assumed to be the major drug-metabolizing organ, responsible for the clearance of most drugs. Drug clearance of an organ is dependent on the blood flow to that organ, the tissue volume of the organ and the extraction ratio of the drug. The liver volume is approxi-

mately 3.9 liters and the blood flow is 1600 ml/min (human). The total skin volume is about 75% of that of the liver (3 liters) with a blood flow only 10% that of the liver (100 ml/min), (Benet, 1978). Unless the extraction ratio of a drug into the skin was very high, the skin would not be expected to play a significant role as an alternate site of drug metabolism.

Since the cutaneous metabolic activity has been shown to be high, it may be possible for these enzymes to exert a first-pass effect (metabolism during absorption) on topically applied drugs. If this drug diffuses slowly through the epidermis, then the skin may serve as a site of first-pass metabolism. Such metabolism may decrease both the amount of drug at the site of action (often the dermis) and the amount systemically available. On the other hand, if absorption is fast, then the cutaneous enzymes may become saturated. In this case, a significant amount of drug may be absorbed into the systemic circulation without being metabolized.

COMPARATIVE IN VIVO STUDIES

The basic data for in vivo human percutaneous absorption, to which animal models are compared, were obtained from Feldmann and Maibach (1969a and b, 1974). In these clinical studies a specific concentration of radioactive compound (4 $\mu g/cm^2$) was applied to a specific anatomical site (ventral forearm); the area was not occluded and subjects were requested not to wash the area for 24 h. The radioactive compounds were applied to the skin in an acetone solution and the acetone quickly evaporated with a gentle stream of air. Urine was collected for 5 days and assayed for radioactivity. A tracer dose was also given parenterally, and the per cent radioactivity in the urine following parenteral administration was then used to correct for compound which might be excreted by some other route and for compound which might be retained within the body.

Bartek et al. (1972) undertook a comparative study of percutaneous absorption in rats, rabbits, miniature swine, and man. Methodology in the animals was similar to that in man except that in animals the compounds were applied to the skin of the back and the site of application was shaved. Radioactive compounds were applied to the skin in the same manner that had been used in man. A non-occluding device was used to keep the animal from removing the applied compound.

Haloprogin, a topical antifungal agent, was completely adsorbed in the rat and rabbit. Penetration through the skin of pigs and man was similar and much slower than it was through rat and rabbit skin. Penetration of acetylcysteine was minimal in all species. Cortisone, a minimal penetrant through the skin of man and miniature swine, was well absorbed in the rat and rabbit. Caffeine readily penetrated the skin of all species. With butter yellow, penetration through rabbit skin was much greater than through the skin of the other 3 species. Testosterone penetration was greatest in the rabbit, followed closely by the rat, and then the pig which was closest to man. The results of this study showed rabbit skin to be the most permeable to topically applied compounds, followed closely by rat skin. In contrast, it appears that the permeability of the skin of the miniature swine is closer to that of human skin (Table 1). Clearly, percutaneous absorption in the rabbit and rat would not be predictive of that in man. It is not known if the subtle differences seen between pig and human skin were due to methodology (site of application, shaving) or

TABLE 1

PERCUTANEOUS ABSORPTION OF SEVERAL COMPOUNDS BY RAT, RABBIT, PIG AND MAN (IN VIVO)

Penetrant	Per cent dose absorbed			
	Rat	Rabbit	Pig	Man
Haloprogin	95.8	113.0	19.7	11.0
Acetylcysteine	3.5	2.0	6.0	2.4
Cortisone	24.7	30.3	4.1	3.4
Caffeine	53.1	69.2	32.4	47.6
Butter yellow	48.2	100.0	41.9	21.6
Testosterone	47.4	69.6	29.4	13.2

the skin itself. However, generally the pig appears to be a good predictor of percutaneous absorption in man.

Bartek and La Budde (1975) also studied the percutaneous absorption of pesticides in the rabbit, pig and squirrel monkey, and compared the results with the absorption obtained in man. The methodology used was the same as their previous studies. The compounds were also applied to the back of the squirrel monkey. The results were compared to man where the site of application was the ventral forearm. DDT was a minimal penetrant in man, whereas in the rabbit and pig penetration rates were considerably greater. Absorption in the squirrel monkey was very low; however, the value reported was uncorrected with parenteral control data. Penetration of lindane, parathion and malathion in the rabbit exceeded that in the other species. With lindane, penetration in the squirrel monkey was closer to that in man, whereas with parathion, penetration in the pig was closest to that in man. Penetration of malathion was similar in the squirrel monkey and the pig, and could be predictive of that in man. It appears that the in vivo percutaneous absorption of pesticides in the rabbit was again much greater than in man, whereas penetration in the pig and squirrel monkey was closer to that in man (Table 2).

Several comparisons of percutaneous absorption in the rhesus monkey and in man were made by Wester and Maibach (1975a and b, 1976, 1977, 1979). Methodology and

TABLE 2

PERCUTANEOUS ABSORPTION OF SEVERAL PESTICIDES BY RABBIT, PIG, SQUIRREL MONKEY AND MAN (IN VIVO)

Pesticide	Per cent dose absorbed			
	Rabbit	Pig	Monkey	Man
DDT	46.3	43.4	1.5	10.4
Lindane	51.2	37.6	16.0	9.3
Parathion	97.5	14.5	30.3	9.7
Malathion	64.6	15.5	19.3	8.2

TABLE 3

PERCUTANEOUS ABSORPTION OF INCREASED TOPICAL DOSES OF SEVERAL COMPOUNDS IN THE RHESUS MONKEY AND MAN (IN VIVO)

Penetrant	Dose ($\mu g/cm^2$)	Per cent of dose absorbed	
		Rhesus	Man
Hydrocortisone	4	2.9	1.9
	40	2.1	0.6
Benzoic acid	4	59.2	42.6
	40	33.6	25.7
	2000	17.4	14.4
Testosterone	4	18.4	13.2
	40	6.7	8.8 *
	250	2.9	
	400	2.2	2.8
	1600	2.9	
	4000	1.4	

* 30 $\mu g/cm^2$.

the site of application (ventral forearm) were the same for both species. The site of application was lightly clipper-shaved in the monkey. A direct comparison of unshaven and lightly clipper-shaved skin showed no difference in absorption (Wester and Meibach, 1975a). Table 3 summarizes the results of these studies. The in vivo percutaneous absorption of hydrocortisone, testosterone and benzoic acid was similar for the rhesus monkey and man. Also, the dose–response was similar in the two species.

In a related study Wester et al. (1977) studied the percutaneous absorption of testosterone in the newborn rhesus monkey. The results showed that the per cent dose absorbed in the newborn rhesus was similar to that in the adult rhesus and adult man. The study also showed that the ratio of surface area to body weight in the newborn is greater than that of an adult. The therapeutic ratio is probably lower in the newborn than in the adult when the compound is applied topically. The newborn rhesus monkey may be a good animal model for studying percutaneous absorption in the neonate.

Andersen et al. (1980) determined the percutaneous absorption of hydrocortisone, testosterone and benzoic acid in the guinea pig and compared the results to man. A concentration of 4 $\mu g/cm^2$ of the ^{14}C-labeled compound was applied to the shaved backs of the animals and percutaneous absorption determined from urinary and fecal excretion. Absorption of hydrocortisone and benzoic acid was similar to published human absorption data. However, testosterone was absorbed to a greater extent in guinea pigs than in man. The absorption value for testosterone in the guinea pig was closer to man if the radioactivity excretion in urine and feces was measured, rather than just the radioactivity excretion in urine alone. If a large proportion of the radioactivity is excreted in the feces, a more accurate estimate of the percutaneous absorption can be obtained by determining the radioactivity excretion in both urine and feces (Wester and Noonan, 1978).

Hunziker et al. (1978) studied the percutaneous absorption of ^{14}C-labeled benzoic acid, progesterone and testosterone in the Mexican hairless dog, and compared the

TABLE 4

COMPARATIVE PERCUTANEOUS ABSORPTION OF TESTOSTERONE IN SEVERAL SPECIES

Species	Per cent of dose absorbed
Rat	47.4
Rabbit	69.6
Guinea pig	34.9
Pig	29.4
Rhesus monkey	18.4
Man	13.2

absorption with that obtained in man. Total absorption and maximum absorption rates were greater in man than in the hairless dog. Surface counting experiments showed that benzoic acid and progesterone persisted on the dog skin far longer than on human skin.

In several of the preceding studies the percutaneous absorption of testosterone was determined. In these studies the same topical concentration, 4 $\mu g/cm^2$, was used. Additionally, the same method of analysis, determination of urinary ^{14}C-excretion, was used. Table 4 summarizes the results. Absorption of testosterone in the rat, rabbit and guinea pig was high compared to man. Absorption in the pig was approximately twice that in man, and that in the rhesus monkey was closest to man. However, it must be remembered that even when the method and applied dose were the same, there were other differences besides species. The site of application in the rat, rabbit, guinea pig and pig was the back, whereas in the rhesus monkey and man the site of application was the ventral forearm. Percutaneous absorption of testosterone in the rhesus monkey and man has been shown to vary with the site of application (Feldman and Meibach, 1967; Meibach et al., 1971; Wester et al., 1980a). What proportions of the variation in the above comparison are due to species and due to site of application is not known. However, it points out that when a difference is found, it could be a sum of the many variables in the study.

In general, the comparative in vivo data which have been reviewed demonstrate that percutaneous absorption in the pig and monkey (rhesus and squirrel) is in most cases similar to that in man, whereas in the rat, and especially in the rabbit, skin penetration is greater than that observed in man. The skin of the Mexican hairless dog has significantly different permeability characteristics than human skin. Absorption in the guinea pig was similar to man for hydrocortisone and benzoic acid, but high for testosterone.

COMPARATIVE IN VITRO STUDIES

Percutaneous absorption can be determined using the in vitro cell diffusion technique. Table 5 summarizes the ranking of skin permeability of different species, as determined in vitro by several investigators (Tregear, 1966; Marzulli et al., 1969; McGreesh, 1965). Allowing for the different compounds used in each study to rank the species and the differences in origin of the skin sample (back, forearm), the studies generally show that the skin of common laboratory animals (rabbit, rat and guinea pig) is more permeable than the skin of man. Skin from the pig and the monkey more generally approximates the

TABLE 5

RANKING OF SKIN PERMEABILITY OF DIFFERENT SPECIES AS DETERMINED IN VITRO; LISTED IN DECREASING ORDER OF PERMEABILITY

Tregear (1966)	Marzulli et al. (1969)	McGreesh (1965)
Rabbit	Mouse	Rabbit
Rat	Guinea pig	Rat
Guinea pig	Goat	Guinea pig
Man	Rabbit	Cat
	Horse	Goat
	Cat	Monkey
	Dog	Dog
	Monkey	Pig
	Weanling pig	
	Man	
	Chimpanzee	

permeability of human skin. Not surprisingly, this general ranking is in close agreement with the in vivo data discussed earlier.

Campbell et al. (1976) investigated the permeation of scopolamine in vitro through rat, rabbit and human skin. The results indicated that human skin is the least permeable of the 3 species tested, and the relative order of rat and rabbit skin permeabilities depends both on skin location (back and side) and the method used to remove the hair.

In the study of Marzulli et al. (1969) mouse skin was the most permeable, and was certainly much more permeable than human skin. In contrast, studies by Stoughton (1975) using human and hairless mouse skin in vitro showed remarkable similarities in absorption for the skin of the two species for many compounds.

Pertinent to the above discussion is the relevance of in vitro absorption data to that obtained in vivo. Franz (1975) evaluated the permeability of 12 organic compounds in vitro using excised human skin and compared the results to those obtained previously by Feldmann and Maibach in living man. Care was taken to ensure that his in vitro conditions closely followed those used in vivo, although it was necessary to use human abdominal skin for the in vitro studies. Additionally, the doses employed ranged from 4 to 40 $\mu g/cm^2$, with the assumption that the per cent of applied dose absorbed would not be dose-dependent. Quantitatively, the in vitro and in vivo data did not agree. The in vitro method was of value to the extent that it tended to distinguish compounds of low permeability from those of high permeability. However, there are notable differences such that the in vitro method alone would not always be a reliable or accurate predictor of percutaneous absorption in living man. A more recent presentation by Franz (1979) suggested that many of the differences in the above studies were due to technical reasons. Future work in this area may prove the in vitro method to be predictive of in vivo absorption.

DISCUSSION

In reviewing studies comparing percutaneous absorption between animals and man, care must be taken to ascertain what influences the methodology may have had on the

data. Differences in results can be due to different techniques used in the study. This becomes very important when the data from an animal study are compared to published literature values on absorption in man. Subtle differences in technology may not be readily expressed in the printed methodology.

When comparing the percutaneous absorption of species it becomes obvious that differences do exist. Some of these differences are due to the species themselves and some of the differences are due to techniques used in the study. Various parameters affect percutaneous absorption. One of these parameters, site of application, was obvious in the preceding review. It becomes important that in any species comparative study, the methods and techniques used must be as close to each other as possible. Some of the parameters such as site of application, occlusion, dose concentration, surface area and vehicle can be controlled by the investigator. Some parameters such as skin metabolism, skin age and skin condition may, in part, be difficult for an investigator to control.

The perfect comparative study probably cannot be done; however, the data in the literature suggest that differences in percutaneous absorption exist between species. Compared to absorption in man, absorption in common laboratory animals, rat and rabbit, is quite high. Absorption in the pig and the monkey (squirrel and rhesus) appears more predictive of that in man. Although absorption in vitro has not been proven to be predictive of that in vivo, the comparative in vitro studies done with skin from different species favorably agree with the in vivo results.

Thus it appears that the animal models most predictive of percutaneous absorption in man are the pig and monkey. As difficult as it may be for an investigator to do an absorption study in man, it may be just as difficult to have access to monkeys and pigs. This then does not mean that the investigator has to do meaningless studies in vitro or in vivo with rats and rabbits. What it means is that the results obtained must be carefully explained within the scope of the methods and species used. Correlations and predictions of results to man must be done with utmost care.

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