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Skin metabolism of topically applied compounds

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Introduction

Drugs can be applied to the skin surface to elicit either local or systemic effects. In the treatment of local skin conditions it may be preferable to concentrate the drug in the outer regions of the skin, while for systemic action penetration into the circulation will be necessary. In both applications, the drug is placed in contact with a metabolically active organ. Clearly drug transformations could take place and may be significantly influenced by the physiological and pathological status of the skin. This may be of particular interest in transdermal drug delivery where a major benefit of this route is considered to be avoidance of first-pass hepatic drug metabolism, which can be a significant factor in the early elimination of a drug from the circulation. Thus, responsibility for first-pass effects is now transferred to the skin. This would normally only play a very minor part in systemic drug metabolism (Benet, 1978), even though specific enzyme activity within the skin approaches and sometimes exceeds that of the corresponding hepatic enzyme (Mauvais-Jarvis et al., 1970; Wester and Noonan, 1980; Rawlins et al., 1980; Noonan and Wester, 1985). Data to support this new metabolic role for skin are seen in bioavailability studies with topical glyceryl trinitrate (Wester et al., 1983) and efficacy studies with topical cortisol (Greaves, 1971). Further, Smith and Holland (1981) and Holland et al. (1984) have suggested that the rate of metabolism of benzo[a]pyrene within the skin is the rate-limiting step when considering percutaneous absorption. Thus, cutaneous metabolism can significantly influence drug delivery through the skin and a study of percutaneous absorption of chemicals should consider the potential for enzymatic modification during transdermal passage in addition to those physicochemical properties which influence drug transfer. Indeed, slow absorption could lead to extensive first-pass metabolism whereas fast absorption could cause saturation of enzyme systems resulting in the availability of large amounts of drug for uptake into the systemic circulation. This review considers the enzymatic potential of the skin in relation to xenobiotic metabolism.

Location of skin enzymes

Notwithstanding its complex nature, skin is normally considered to consist of 3 distinguishable regions: the epidermis, the dermis and the hypodermis. The epidermis, the outermost region of the skin, consists of a basal layer of actively dividing cells which slowly progress outwards until they form the dead, flat, keratinised cells of the stratum corneum. The stratum corneum represents the major barrier to percutaneous absorption for the

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majority of compounds. The dermis, underlying the epidermis, is a connective tissue containing many nerve fibres and blood vessels which supports and nourishes the avascular epidermis. The hypodermis is an insulating layer of connective tissue containing many adipose cells.

Montagna (1955), in histological investigations, first reported on the localization and activity of skin enzymes in the epidermis and also offered evidence of the metabolic capacity of the hair follicles, sebaceous glands and apocrine sweat glands. Further evidence has since established the epidermis as the major site of metabolism in the skin of man (Bamshed, 1969; Laerum, 1969; Yu et al., 1979; Bickers et al., 1982) and other species (Meyer and Neurand, 1976; Yu et al., 1980a). Indeed, esterase activity has been shown to be 4–9 times higher in the epidermis than the dermis of hairless mouse skin (Yu et al., 1980b); similarly, 96.5% of aryl hydrocarbon hydroxylase activity was found located in the epidermis (Chapman et al., 1979). It is worth commenting, however, that it can be misleading to compare activity per unit tissue mass because of the difference in dermal and epidermal skin content. Finnen et al. (1985) illustrated this by comparing enzyme activity per unit area and showed that using this criteria activity within the dermis is about 5 times greater than in the epidermis. The major argument against the role of the dermis in metabolism is the short residence time of most compounds within this region. The capillary loops are known to come very close to the dermoepidermal junction ensuring that applied compounds usually only remain in the dermal layer for a short time before being taken up into the general circulation, therefore having minimal contact with the dermal enzymes. It could be argued that extracellular fluid originating from the dermal layer via the normal circulatory flow could carry enzymes to the skin surface, causing the dermal enzymes to have some role to play in xenobiotic metabolism. The accessibility of enzymes to the drug is obviously important since the chemical structure of a compound will determine whether drugs will penetrate into cells, whereas extracellular enzymes will have a greater effect on all susceptible topical compounds (Hansch, 1972). This may be particularly relevant when considering situations where the barrier is compromised, e.g. broken skin.

Subcellularly, enzyme activity seems mainly associated with the cytoplasmic membrane (Akin and Norred, 1976), being found predominantly in the smooth microsomal fraction (Finnen et al., 1985) although some cytosolic enzymes have been isolated (O'Neill and Carless, 1980; Pannatier et al., 1981b).

Individual skin cells possess enzymes associated with primary catabolic and anabolic metabolism. For example, skin has been shown to contain the enzymes integral to carbohydrate metabolism (Pillsbury, 1931; Cruickshank et al., 1957; Halprin and Ohkawara, 1966a; Mier, 1969; Johnson and Fusara, 1972; Kondo and Gerna-Torsellini, 1974). It seems that anaerobic metabolism predominates giving the major end product of lactate (Freinkel, 1966; Decker, 1971), although Halprin and Ohkawara (1966c) do suggest that lactate dehydrogenase, the enzyme responsible for turning pyruvate into lactate, works only at much reduced capacity in the skin. Further details can be found in a review of the subject by Freinkel (1983). Skin also produces fatty acids (Nicolaides, 1974), oxidising them as an energy source (Freinkel, 1966; Decker, 1971), is involved in protein synthesis (Rothberg et al., 1961; Freedberg and Baden, 1964; Freedberg, 1972) and is active in the production of vitamin D (Holick et al., 1980, 1981).

Xenobiotic metabolism

An extensive literature exists to indicate the capacity of the skin to metabolise many compounds. It seems likely that the skin can participate in both Phase I (functionalization) reactions like oxidation, reduction and hydrolysis and Phase II (conjugation) reactions, like glucuronide and sulphate conjugate formation. Some principal reactions and the enzymes involved in these xenobiotic transformations are summarised in Table I.

Skin metabolism has been shown to contribute significantly to the activation of potential carcinogens. For instance, compounds such as the polycyclic hydrocarbons must form electrophilic moieties before they become carcinogens (Sims and Grover, 1974; Gelboin, 1977) and enzymes

TABLE 1

Some principal reactions and the enzymes involved in cutaneous xenobiotic reactions

Reaction type	Selected transformations	References *
Phase I		
Oxidation	*17β-hydroxysteroid dehydrogenase*	
	testosterone → androstene-3,17-dione (DHT)	11
	hydrocortisone → cortisone	14
	17β-oestradiol → oestrone	29
	mixed-function oxidase (aryl hydrocarbon hydroxylase)	
	7,12-dimethylbenz[a]anthracene → 7-hydroxy-12-methylbenz[a]anthracene	5
	dehydroepiandrosterone → 7α/7β-hydroxydehydroepiandrosterone	6
	Benzo[a]pyrene → various phenols, quinones and dihydrols	8, 16, 21
	7-ethoxycoumarin deethylase	
	7-ethoxycoumarin → coumaric acid	10
	monoamine oxidase	
	noradrenaline → dehydroxymandelic acid	12
	DOPA decarboxylase	
	DOPA → dopamine	13
	dopamine-β-oxidase	
	dopamine → noradrenaline	13
	aniline hydroxylase	
	aniline → *p*-aminophenol	22
	adenosine deaminase	
	vidarabine → β-D-arabinofuranosyl hypoxanthine	30
Reduction	*ketoreductase*	
	progesterone → pregnonediol	9
	hydrocortisone → reichsteins	14
	phorbolmyristate acetate → phorbololmyristate acetate	25
	oestrone → 17β-oestradiol	29
	5α-reductase	
	testosterone → 5α-dihydrotestosterone	9
	progesterone → 5α-pregnane derivatives	9
	hydrocortisone → allodihydrocortisol	14
Hydrolysis	*epoxide hydratase*	
	styrene oxide → styrene glycol	2
	esterases	
	hydrocortisone-17 esters → hydrocortisone	19
	hydrocortisone-21 esters → hydrocortisone	19
	betamethasone-17-valerate → betamethasone	23
	diflucortolone valerate → diflucortolone	28
	acetylsalicylic acid → salicylic acid	17
	benzoylperoxide → benzoic acid	18
	p-nitrobenzoate esters → *p*-nitrobenzoic acid	21
	cromoglycate esters → cromolyn	4
	theophylline esters → theophylline	26
	naphthyl esters → naphthol	7
	metronidazole esters → metronidazole	15
	pseudomonic acid → monic acid	1
	Aryl ester-o-dealkylase	
	p-nitroanisone → *p*-nitrophenol	20
	p-nitrophenetol → *p*-nitrophenol	20

TABLE 1 (continued)

Reaction type	Selected transformations	References *
Phase II		
Methylation	*catechol-o-methyl transferase*	
	noradrenaline → normetanephrine	12
Glucuronidation	UDP-glucuronyl transferase	
	p-aminophenol → *p*-aminophenol glucuronide	24, 27
	p-nitrophenol → *p*-nitrophenol glucuronide	24, 27
Sulphatation	dehydroepiandrosterone → dehydroepiandrosterone sulphate	3
	Δ5-androstene-3β-17β-diol → Δ5-androstene-3β,-17β-diol sulphate	3

* References:

1 Baines et al., 1984
2 Bentley et al., 1976
3 Berliner et al., 1968
4 Bodor et al., 1980
5 DiGiovanni et al., 1977
6 Faredin et al., 1969
7 Findlay, 1955
8 Fox et al., 1975
9 Frost et al., 1969
10 Goerz et al., 1981
11 Gomez and Hsia., 1968
12 Håkanson and Moller, 1963a
13 Håkanson and Moller, 1963b
14 Hsia et al., 1965
15 Johanssen et al., 1986
16 Kao et al., 1983
17 Loftsson., 1982
18 Nacht et al., 1981
19 O'Neill and Carless, 1980
20 Pannatier et al., 1981a
21 Pannatier et al., 1981b
22 Pohl et al., 1976
23 Rawlins et al., 1979
24 Rugstad and Dybing., 1975
25 Segal et al., 1975
26 Sloan and Bodor., 1982
27 Stevenson and Dutton., 1960
28 Tauber and Toda, 1976
29 Weinstein et al., 1968
30 Yu et al., 1981

such as aryl hydrocarbon hydroxylase play an important role in this activation process. An example of this is the transformation of the inactive benzo[a]pyrene to the carcinogenic benzo[a] pyrene-7,8-diol-9,10-epoxide which then interacts with cellular DNA (Sims et al., 1974).

Metabolism in specialised skin structures

The sebaceous glands, eccrine sweat glands and hair follicles present a limited capacity for metabolism, although little work has been done to assess their contribution to skin metabolism as a whole. They cannot, however, be essential since metabolism still occurs in the sole in the foot where these structures are absent (Hsia et al., 1965).

The sebaceous glands are dependent on stimulation by androgens for growth and activity and appear to be involved in steroid metabolism (Milne, 1969). Work has shown that the glands have high 5α-reductase activity, metabolising testosterone to 5α-dihydrotestosterone (Bardin et al., 1973; Sansone-Bazzono and Reisner, 1974). In the skin of the forehead about 50% of this enzyme and 90% of the enzyme 3B-hydroxysteroid dehydrogenase-4,5-isomerase is found in the sebaceous gland (Hay and Hodgins, 1978). Coomes et al. (1983) have shown that for a limited range of enzymes the sebaceous glands are the most active structures within the skin. Sebaceous glands have also been found to contain the enzymes for glycogen metabolism, active glycolysis and amino-acid metabolism (Im and Hoopes, 1974). Similarly, it has been shown that all lactate found in sweat can be accounted for by glandular, rather than general, skin metabolism (Wolfe et al., 1970). Lipogenesis also occurs (Fazekas and Sandor, 1972) although the evidence suggests that squalene is the only major product. Thus, it seems that the sebaceous glands are the major site of squalene synthesis within the skin (Nicolaides and Rothman, 1954). Eccrine sweat has been shown immunologically to contain a wide range of esterases (Hermann and Habbig, 1976).

Hair follicles are also responsive to androgens since events such as growth of beard and male baldness are dependent on levels of circulating androgens. Isolated hair follicles metabolise testosterone by 5α-reduction and 17β-hydroxysteroid dehydrogenation (Greismer and Thomas, 1963; Fazekas and Sandor, 1972; Takayasu and Adachi, 1972; Schweikert and Wilson, 1974), although there are variations in androgen metabolism between different skin sites (Takayasu and

Adachi, 1972). No significant relationship has been found between capacity for metabolism and any of the androgen-mediated growth patterns of hair (Schweikert and Wilson, 1974). If it could be shown that metabolism in hair follicles reflects that in other parts of the body, a useful method of testing enzyme activity could be developed since hair follicles are readily accessible. This possibility was first considered by De Bruyn et al. (1977) looking at detection of galactosaemia by studying galactose metabolism in hair follicles.

Metabolism in diseased and healthy skin

Several authors have suggested a relationship between metabolic changes and skin disease states, although it is not known whether these changes are a result or the cause of observed diseases. For example, in psoriasis, a disease characterised by overproliferation of basal cells, the enzymes associated with cell multiplication are increased (Rippa and Vignali, 1965; Halprin and Ohkawara, 1966b; Kondo and Gerna-Torsellini, 1974), and this was seen as an effect rather than the cause of the disease. However, Chapman et al. (1977) showed that aryl hydrocarbon hydroxylase activity was much reduced in both involved and non-involved skin of psoriatics compared with normal patients leading to the suggestion that skin metabolism could be important in the initiation of disease states. They also showed that the aryl hydrocarbon hydroxylase in the psoriatic epidermis cannot be induced as in normal epidermis. Previous work had shown alterations in adenylate cyclase activity and imbalances in cyclic guanine monophosphate to cyclic adenylate monophosphate ratios in psoriatic skin (Voorhees et al., 1973; Mui et al., 1975).

Another skin disorder where metabolism has been found to be abnormal is acne vulgaris. Sansone and Reisner (1971) reported that the skin of acne patients produces 2–20 times more of the testosterone breakdown product dehydrotestosterone than the skin of normal volunteers. Reinforcing these findings, other workers have found increases in other enzymes associated with steroid metabolism (Baillie et al., 1966; Hay and Hodgins, 1974; Cooper et al., 1978).

Finally, skin metabolism may be altered due to disease states which do not directly affect the skin. For example, several groups have shown metabolism of steroids by males with testicular feminization syndrome to be altered. Here skin seems to lack the 5α-reductase which turns testosterone into dehydrotestosterone (Mauvais-Jarvis et al., 1969b; Wilson and Walker, 1969) although 5α-hydrogenation of progesterone still occurs (Mauvais-Jarvis et al., 1969a).

Enzyme induction and inhibition

In common with hepatic enzymes, it seems that cutaneous enzymes are prone to induction and inhibition. For example, coal tar, containing many polycyclic hydrocarbons, was shown to induce aryl hydrocarbon hydroxylase in vivo and in vitro to varying degrees (Bickers and Kappas, 1978) and the compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin can increase aryl hydrocarbon hydroxylase activity by up to 30-fold over a 75-h period (Pohl et al., 1976). In contrast, it has been shown that the metabolism of 7,12-dimethylbenz[a]anthracene is inhibited by compounds such as 17β-estradiol and 5,6-benzoflavin (DiGiovanni et al., 1977) the latter compound having been shown to reduce both aryl hydrocarbon hydroxylase activity and its induction (Bowden et al., 1974). This subject has been extensively reviewed by Noonan and Wester (1985).

Applications of metabolism in topical therapy

One way in which the metabolic capacity of the skin can be exploited in the field of transdermal drug delivery is with the use of prodrugs and soft drugs (Bucks, 1984).

Prodrugs are defined as applied inactive compounds which are enzymatically transformed into the active form at or near their site of action. This is of particular value where the active form has low penetration capability. Many examples of this approach can be found in the literature. For example, Boder et al (1982) successfully developed lipophilic prodrugs of the polar anti-inflammatory agent cromoglycic acid (cromolyn). Cromolyn, which penetrates the skin poorly, showed increased passage when employed as a prodrug, the best results being obtained with the hexanolyloxyethylidene and hexanolyloxymethyl esters.

Sloan and Bodor (1982) have also utilised the same approach using theophylline as an anti-psoriatic agent. The hydroxymethyl and acyloxymethyl prodrugs of theophylline give increased penetration of theophylline into skin. Johanssen et al. (1986), employing metronidazole esters as delivery systems for metronidazole, showed that no single ester offered optimal properties regarding both skin permeation and enzymatic transformation, although they concluded that the butyrate ester seemed to fulfill best the relevant characteristics. Optimal structures will depend on the relative rates of diffusion and metabolism of the drug.

Soft drugs are active compounds which become inactivated in the skin. Whilst this is generally a disadvantage, it may be considered useful if the compound is likely to be systemically toxic, since inactivation in the skin after it has acted but before it reaches the circulation may then limit toxicity. Examples of this are found in steroid therapy (Kapp et al., 1977) and in the use of benzoyl peroxide, which breaks down to benzoic acid in skin, for the treatment of acne vulgaris (Nacht et al., 1981).

The effect of skin organisms

A topically applied compound will also encounter the micro-organisms found on both healthy and diseased skin. Such organisms have been shown to be capable of metabolising drugs such as betamethasone-17-valerate (Brookes et al., 1982) and glyceryl trinitrate (Denyer et al., 1984). Analysis of the results using a mathematical predictive model has suggested that microbial degradation of drugs could possibly have an effect on compounds applied to the skin surface as a thin film (Denyer et al. 1985). This may become of more importance with the proliferation of transdermal delivery systems. Such devices may be left on the skin surface for periods of up to one week and could provide an environment in which the micro-organisms can flourish.

Discussion

The application of drugs to the skin surface is an established technique for topical disorders but its utilisation for systemic drug delivery is a relatively new concept. The advantages of percutaneous delivery have been described by Shaw and Chandrasekaran (1978) and include avoidance of variable absorbance rates associated with oral delivery, improved efficacy of lower daily dosing regimes resulting from continuous drug input, and bypassing of first pass hepatic metabolism. It must be remembered, however, that the skin is metabolically active and capable of xenobiotic transformation and this could influence the behaviour of susceptible drugs. It is important to realise that the true extent of such problems may not be revealed using conventional models for studying skin penetration. Kao et al. (1985) using skin maintained as short term organ culture showed extensive 'first-pass' metabolism of both testosterone and benzo[a]pyrene which had previously been undetected using non-viable skin preparations. Considerable variation exists in the structure and properties of skin in health and disease, between individuals and body sites. Cutaneous metabolism is also influenced by those factors which affect systemic biotransformations in general such as age, sex, race and hormonal status, and this will account for considerable intersubject variation in metabolic potential (Chapman et al., 1979). In addition, we must also recognise the capacity for enzyme induction and inhibition by applied chemicals which may lead to a drug transformation of potential toxicity or the precipitation of a disease state. Many preliminary experiments have been conducted using animal models, but in these the enzyme distribution and activity may well be completely different from that in humans and could be misleading.

Conclusion

In the past, skin penetration studies have usually concentrated on the physicochemical factors affecting the passage of chemicals through the skin and have largely ignored the metabolic potential of this organ. More recently evidence from in vitro and in vivo experiments has shown that cutaneous metabolism may significantly influence local drug action, toxicity and delivery through

the skin. It is necessary therefore to establish the susceptibility to enzymic modification of drug candidates intended for topical use, in order to assess more fully their suitability for this form of drug delivery and for application to diseased or abraded skin, where the enzyme profile may differ. Furthermore, an appreciation of enzyme location and function may assist in the development of prodrugs with optimal properties to aid percutaneous penetration.

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