Review Article

Skin Pharmacology and Physiology

Skin Pharmacol Physiol 2019;32:283–293
DOI: 10.1159/000501732

Received: March 17, 2019
Accepted after revision: June 25, 2019
Published online: July 29, 2019

Skin Metabolism: Relevance of Skin Enzymes for Rational Drug Design

Sung Min Pyo[a] Howard I. Maibach[b]

[a]Department of Biology, Chemistry, and Pharmacy, Freie Universität Berlin, Berlin, Germany;
[b]Department of Dermatology, University of California School of Medicine, San Francisco, CA, USA

Keywords
Skin metabolism · Skin enzymes · Transdermal therapeutic systems · Dermal delivery systems · Dermal prodrugs · Dermal soft drugs · Stereoselective metabolism

Abstract
Transdermal therapeutic systems (TTS) have numerous pharmacological benefits. Drug release, for example, is independent of whether a patient is in a fed or a fasted state, and lower doses can be given as gastrointestinal and hepatic first-pass metabolism is avoided. In addition, inter- and intrapatient variability is minimized as the release of the drug is mainly controlled by the system. This makes TTS interesting as alternative systems to the most common dosage form of oral tablets. The difficulty with the dermal administration route is transporting the drug through the skin, since the skin is an efficient barrier against foreign bodies. Various strategies have been reported in the literature of how drug penetration can be improved. Most of them, however, focus on overcoming the stratum corneum as the first (mechanical) skin barrier. However, penetration is much more complex, and the skin's barrier function does not only depend on the stratum corneum; what has been underestimated is the second (biological) skin barrier formed of enzymes. Compared to the stratum corneum, very little is known about these enzymes, e.g., which enzymes are present in the skin and where exactly they are localized. Hence, very few strategies can be found for how to bypass or even use the skin enzyme barrier for TTS development. This review article provides an overview of the skin enzymes considered to be relevant for the biotransformation of dermally applied drugs. Also, we discuss the use of dermal prodrugs and soft drugs and give the stereoselectivity of skin metabolism careful consideration. Finally, we provide suggestions on how to make use of the current knowledge about skin enzymes for rational TTS design.

1 Introduction

1.1 Transdermal Therapeutic System

A drug can be administered via various administration routes. The most common dosage form in pharma worldwide is the single-dose oral tablet. Recently, there has been a trend towards alternative dosage forms and administration routes. Especially the market share of trans-

E-Mail karger@karger.com
www.karger.com/spp

Sung Min Pyo
Institute of Pharmacy, Department of Biology, Chemistry, and Pharmacy
Freie Universität Berlin, Kelchstrasse 31
DE–12169 Berlin (Germany)
E-Mail pyo.sungmin@fu-berlin.de

dermal therapeutic systems (TTS) has grown significantly. In 2005, the global TTS market was valued at USD 3 billion and a compound annual growth rate (CAGR) of 12% was predicted [1]. Ten years later (in 2016), the predicted CAGR was confirmed: the TTS market value had increased 10-fold to USD 30 billion [2]. More than 50% of the total volume was generated in North and Latin America, followed by Europe with 30%, and Asia Pacific with 25%. The TTS market value continues to rise. The CAGR is predicted to reach 7.5% by 2024 [3].

One explanation for this tendency might be that TTS make use of a convenient route for drug administration. TTS are small and lightweight, have a high durability, and can be stored at room temperature. Also, no water or other supplies are needed for their use. Patients therefore show high compliance with TTS treatment [4]. From a pharmacological view, a TTS overcomes potential disadvantages of oral dosage forms and can therefore act as a good complement to single-dose tablets. For example, drug release is independent of whether a patient is in a fed or a fasted state. Also, in most cases, a lower drug dose can be given compared to oral dosage forms, as gastrointestinal and hepatic first-pass metabolism is avoided. Constant plasma drug concentrations are achieved, without the typical plasma concentration peaks after each tablet intake. In addition, inter- and intrapatient variability is minimized as the release of the drug is mainly controlled by the system. For these reasons, TTS are a good and promising addition to the standard dosage form of the tablet.

1.2 Penetration and Permeation of Dermally Applied Drugs

To better understand the penetration and permeation mechanisms of dermally applied drugs, it is important to first understand the physiological functions of the skin. Its main functions are (a) protection against invading pathogens and other foreign substances from the external environment and (b) retaining water and essential nutrients. Both functions depend on a strong barrier function of the skin. Basically, the skin possesses two different barriers: a physical and a biochemical barrier. A drug must overcome both barriers, whereas the skin cannot distinguish between good and bad foreign substances.

1.3 The Skin as a Highly Efficient Barrier

1.3.1 The Stratum Corneum as the First (Physical) Barrier

The stratum corneum (SC) is the outermost part of the human skin. Being in direct contact with the environment, the SC can be counted as the first barrier of the body. It generally consists of 10–15 layers of highly ordered corneocytes. In a dry state, the layers of the SC are around 10–15 μm thin, whereas in a hydrated state the corneocytes absorb water and the thickness of the SC increases significantly to up to 40 μm. Lipids produced by keratinocytes cover the corneocytes. This lets the environment of the SC alternate between hydrophilic and lipophilic [5]. Therefore, besides other properties, foreign substances particularly need a well-balanced lipophilic and hydrophilic profile to overcome this barrier.

1.3.2 Skin Enzymes as the Second (Biochemical) Barrier

Foreign substances not trapped by the first barrier can be biotransformed by enzymes of the viable epidermis and dermis. The metabolic process may increase the water solubility of foreign substances to achieve an increased excretion and elimination rate. Biotransformation, therefore, may serve as an explanation for why efficient drugs show only a decreased or almost no effect after penetration [6]. With respect to the number of cells, the skin possesses a biotransformation activity of one-third of that of the liver. Therefore, the skin is an organ with a high enzyme activity, which is important to consider when designing dermally administered drugs. Currently, however, little is known about skin metabolism.

1.4 Aim

This review article provides an overview of enzymes reported to be present in human skin. Enzymes considered to be highly relevant for the biotransformation of dermally applied drugs are highlighted and described in detail. Also, the use of skin prodrugs and soft drugs is discussed, and stereochemical considerations are provided. Suggestions are made on how to make use of the current knowledge about skin metabolism for rational dermal drug design.

2 Results

2.1 Why Does Biotransformation Occur in Human Skin?

Biotransformation in the skin has the aim to make penetrating foreign substances less active and more water-soluble for better excretion and elimination. For this, the skin metabolizes foreign substances in two consecutive phases: a functionalization phase and a conjugation phase. In the functionalization phase, a polar group is ei-

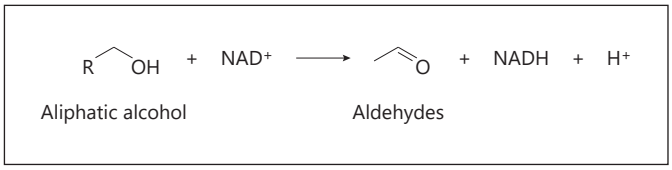

Fig. 1. Oxidation of aliphatic alcohol to aldehydes catalysed by alcohol dehydrogenase.

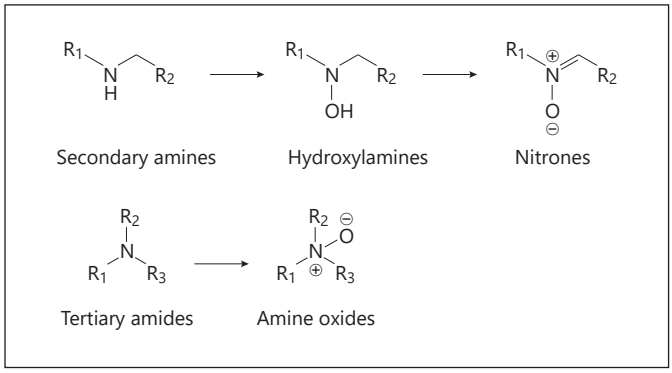

Fig. 2. N-oxidation of tertiary and secondary amines catalysed by flavin-dependent monooxygenase.

ther generated or unmasked by oxidative, reductive or hydrolytic reactions. In the second phase, the so-called conjugation phase, small hydrophilic endogenous molecules, e.g., glucuronic acid, sulphate or glycerine, are covalently attached. The reaction product will always have an increased molecular weight. Nevertheless, excretion is improved due to pronouncedly increased hydrophilicity.

It is well known that enzymatic biotransformation of foreign substances is most effective in the human liver. Preliminary investigations, however, have shown that both the types of enzymes and the enzyme activities are not directly transferable to the skin; thus, knowledge of skin metabolism has to be acquired separately. So far, little is known and very little information is available. In the following, we list a selection of enzyme families. They have both been reported to be present in human skin and been shown to be considerably involved in the degradation of topically applied drugs.

2.1.1 Alcohol Dehydrogenase

In humans, 5 enzyme classes of the alcohol dehydrogenase (ADH) family are known: ADH1 with subunits α, β and γ, ADH2, ADH3, ADH4 and ADH5 [7]. The most prominent representative of the ADH family is ADH1, catalysing the oxidation of primary and secondary aliphatic alcohols to aldehydes (Fig. 1).

The main aim of this biotransformation is the detoxification of penetrating foreign substances. Ethanol, for example, is detoxified by liver ADH1 to ethanal. However, also the opposite can occur; methanol is metabolized by the identical enzyme to toxic formaldehyde. Hence, these two phenomena can also emerge after drug administration. Biotransformation can make drugs inactive, more potent or even toxic. This fact makes the investigation of skin enzyme activities on drugs relevant.

ADH was found in both human keratinocytes and hair root cells [8]. ADH activity on various alcohols in human skin was compared in vitro on excised human skin via enzyme assays, and in vivo on 12 clinically normal subjects via an acute patch test [9]. In vitro, enzyme activity was expressed by the amount of generated NADH (Fig. 1), and in vivo by the occurrence of erythema. The activity of ADH varied for the different alcohols. The biotransformation rate increased clearly with an increasing length of the carbon chain. For example, pentanol (C5) was 3 times more catalysed by ADH1 than ethanol (C2). In addition, with an increasing degree of branching, the biotransformation rate decreased. Hence, the biotransformation rate of alcohols depends on the length and degree of branching: an increasing length of the carbon chain and a decreasing degree of branching lead to higher metabolic biotransformation.

2.1.2 Flavin-Dependent Monooxygenase

Flavin-dependent monooxygenase (FMO) is the main enzyme class for oxidation [10]. In human skin, it has a high transcription level, even higher than that of the CYP family [11]. The main difference between the two is how oxidation proceeds; CYP uses an oxygenated haem prosthetic group, whereas FMO uses flavin adenine dinucleotide for substrate oxidation. FMO mainly biotransforms amine-, sulphide-, phosphorus-, and other nucleophilic heteroatom-containing compounds (Fig. 2, 3).

In the human body, 5 isoforms exist. Which of the 5 isoforms are expressed in skin is controversially reported. Hu et al. [12] found all 5 isoform transcripts in native human skin, whereas Jäckh et al. [13] only observed transcription of FMO1 and FMO3. The presence of FMO3 was confirmed also by Wilkin and Stewart [9], via immunoblot analysis. However, no FMO1 was detected. Comparable findings were reported by Vyas et al. [14]. Thus, so far, in human skin only the transcription of FMO3 seems to be definitively proven. Using benzydamine as a model substrate, the activity of FMO enzymes was as-

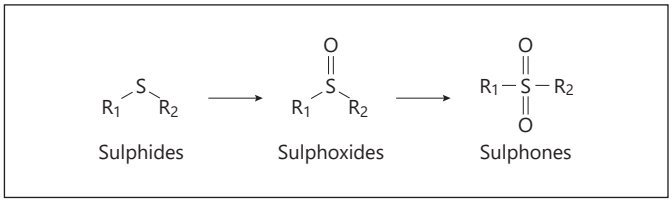

Fig. 3. S-oxidation of sulphides to sulphones catalysed by flavin-dependent monooxygenase.

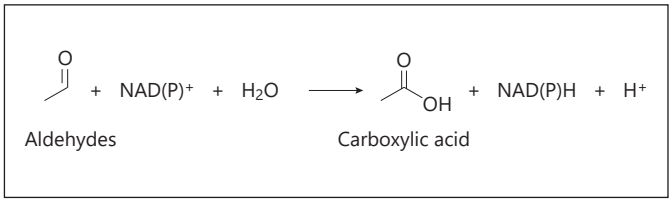

Fig. 4. Oxidation of aldehydes to carboxylic acids catalysed by aldehyde dehydrogenase.

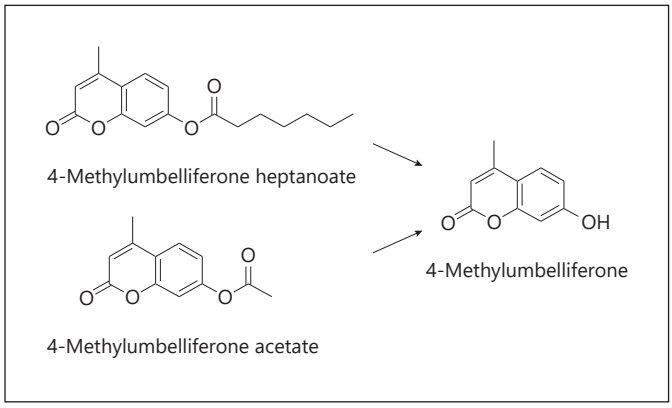

Fig. 5. Ester hydrolysis of 4-methylumbelliferone heptanoate and acetate to 4-methylumbelliferone catalysed by carboxylesterase.

sessed in microsomes of abdominal skin biopsies. FMO biotransformed benzydamine to benzydamine N-oxide with a high oxygenation activity level of 5 nmol/min/mg protein [15]. This value strengthens the assumption that FMO is involved as the main enzyme class in the oxygenation of dermally applied drugs.

2.1.3 Aldehyde Dehydrogenase

Aldehyde dehydrogenase (ALDH) converts aldehydes into carboxylic acids (Fig. 4). Twelve classes of ALDH were found in the entire human body [16]. In excised human skin, only ALDH1 and ALDH3 were detected, by Western blot analysis and immunohistochemistry. ALDH1 has a high affinity for aldophosphamide and plays an important role in the detoxification of peroxide aldehydes. In contrast, ALDH3 preferentially oxidizes aromatic aldehydes and medium-chain aliphatic aldehydes (fatty aldehydes). Compared to the activity of ALDH in the liver, its activity in skin is reported to be 22-fold lower [8]. In addition, variation in ALDH activity according to gender and/or anatomical site was reported. Besides these facts, only little is known about ALDH in skin.

2.1.4 Carboxylesterase

Carboxylesterase (CE) hydrolyses carbon esters by intramolecular addition of water to an alcohol and acidic residue: carboxylic ester + H2O ⇌ alcohol + carboxylate. Alcohols are then further oxidized to aldehydes and the carboxylates conjugated in phase 2 of biotransformation. The CE family is organized into 5 classes [17]. In the human liver, the isoenzymes CE1 and CE2 were found [18, 19]. In human skin, only the presence of the CE2 isoenzyme has so far been verified in human keratinocytes [20]. Besides the cytochrome P450 (CYP) enzymes, the CE enzyme family are one of the more rigorously investigated skin enzymes. This reflects the importance of this family for the biotransformation of dermally applied drugs and for detoxification of foreign substances.

CE activity has been assessed in homogenates of human keratinocytes using fluorescein diacetate as a model substrate [21]. A hydrolytic activity of 3.7 nmol/min/mg protein was measured. Using p-nitrophenyl acetate as a model substrate instead, a much higher hydrolytic activity was measured: 45 nmol/min/mg protein was hydrolysed in cytosolic human skin fractions [22], corresponding to a 12-times higher hydrolytic activity.

Interindividual fluctuation of hydrolytic activity was determined in primary breast keratinocytes of skin donors. Biotransformation of the model drug 4-methylumbelliferone heptanoate distinctly varied from 10 to 32 nmol/min/mg protein [23]. Therefore, there was a highly polymorphic CE expression. Using 4-methylumbelliferone acetate instead of the heptanoate, a much weaker hydrolysis rate of 0.5 nmol/min/mg protein was measured [24]. The only difference between the two molecules lies in their chain lengths (C2 and C7). In analogy to ADH, the molecule with the longer carbon chain length was biotransformed at a higher rate (Fig. 5).

2.1.5 Cytochrome P450

CYPs are the largest group of metabolizing enzymes in skin. They catalyse the transfer of one oxygen atom of

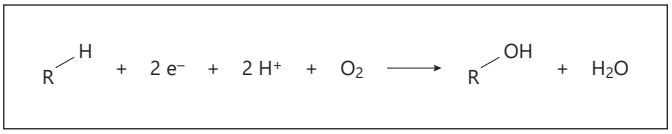

Fig. 6. Cytochrome P450-catalysed transfer of one oxygen atom resulting in alcohol and water.

molecular oxygen onto penetrating substrates, generating alcohol and water (Fig. 6).

Expression of CYP appears to be polymorphic [10]. Its activity shows remarkable interindividual variability depending on age, gender and the anatomical site of application. Which of the CYP enzymes are present in human skin has still not been clarified; contradictory data were reported. Hu et al. [12] and Hayden et al. [25] both found CYP2D6, CYP2E1 and CYP3A4 transcription in native human skin. In contrast, CYP1A1/2 and CYP2C9 were only demonstrated by Hu et al. [12], and CYP1B1 and CYP2A6 only by Hayden et al. [25].

2.2 Where in the Skin Does Biotransformation Take Place?

To verify the location of biotransformation, the hydrolysis of prednisolone (PS) 21-acetate to PS was measured [26]. Excised human skin was either separated into epidermis and dermis by immersing the skin in water at 60 °C or cut into 10-µm-thick slices after freezing.

The following hydrolytic activities were found:

- Whole skin: 0.69 nmol/min/mg protein/mm thickness
- Dermis: 0.41 nmol/min/mg protein/mm thickness
- Epidermis: 5.2 nmol/min/mg protein/mm thickness

Hydrolysis, therefore, was proven to occur mainly in the epidermis. Analysis of the skin slices allowed for a more precise localization: of the entire skin, the stratum basale showed the highest hydrolytic activity [26].

In that study, however, the skin was either heated or frozen during the investigation. Enzymes are thermolabile. Especially when heated above 40 °C, enzymes suffer a considerable loss of activity. An elegant method for the investigation of the hydrolytic activity of the respective skin layers seems to be confocal laser scanning examination. With this method, activity localization is possible without having to separate the skin into layers by heating or freezing. Fluorescein-5-isothiocyanate diacetate is applied to excised human skin and allowed to penetrate. If esterase enzymes are present, hydrolysis occurs and the green fluorescent fluorescein-5-isothiocyanate is released. This reaction product can then be visualized by confocal laser scanning. Only the viable epidermis emitted a green glow, indicating that the viable epidermis is the most enzymatically active part of the skin [27].

The disadvantage of both abovementioned methods is the need for a model substance. As mentioned in the previous section, the activity of an enzyme is substance specific. Also, in both cases, only the enzymes possessing hydrolytic activity were investigated. One method that avoids using a model substance and represents the activity of all enzymes is the measurement of oxygen consumption. It has been assumed that oxygen consumption is proportional to the enzyme activity of cells. The viable epidermis showed the highest oxygen consumption at 4.53 ± 1.39 µL O_2/mg/h. At a far lower level, the dermis consumed 0.49 ± 0.12 µL O_2/mg/h, which is only about one-tenth of the oxygen consumption of the viable epidermis under identical conditions [28].

No matter how different the three methods are, they all lead to the same conclusion: the viable epidermis is the main location of biotransformation. The dermis also shows enzymatic activity, but it is much weaker than that of the epidermis. In addition, in the dermis the residence time of drugs is usually short, due to uptake into the systemic circulation by capillaries. In sum, relevant drug biotransformation takes place almost exclusively in the viable epidermis.

2.3 Factors Influencing the Enzymatic Activity of the Skin

2.3.1 Age

Esterase enzymes, mainly located in the keratinocytes of the skin, play an important role in skin metabolism. Therefore, the development and growth processes of keratinocytes are directly connected to its esterase activity. Ngawhirunpat et al. [29] found that when growing keratinocytes, esterase enzymes are also increased. The biotransformation capacity of skin is age related. This should be considered when highly metabolized drugs are applied dermally, since on children, usually no clinical data are collected for dose recommendations; evaluated and well-known doses for adults are often simply reduced according to the weight of the child. For drugs highly metabolized in skin, this method for dose adjustment cannot be recommended.

2.3.2 Anatomical Site and Gender

Even though the skin is considered as one organ, the requirements for each part of the skin are different. As a result, some enzymes are only found in specific regions of the body. The most prominent example is hydrocortisone

5α-reductase; activities of this enzyme were only found in the foreskin and scrotal skin [30]. Thus, this example also reveals a gender-specific expression of skin enzymes. No further literature was found dealing with the gender-specific activities of skin enzymes. However, sex differences found in hepatic metabolism suggest that differences may also exist in skin metabolism.

2.3.3 Environmental Factors

The activity of liver enzymes is well known to be dependent on external factors. Skin enzymes also show this property. Environmental factors widely described in the literature as influencing enzyme activity are UV radiation and exposure to air pollution. UV radiation itself does not activate or inactivate enzymes, but it generates reactive oxygen species. An increased level of reactive oxygen species, for example, leads to the suppression of matrix metallopeptidase inhibitors, and thereby to increased MMP activity in the skin [31]. Further examples of UV radiation-inducible enzymes in the skin are the two CYP enzymes CYP1A1 and CYP1B1 (see Section 2.1.5) [32], haem oxygenase [33, 34], cyclooxygenase [33, 35] and nitric oxide synthase [36].

Furthermore, modification of the skin enzyme level by air pollution has been discussed in the literature. On the surface of particulate matter, polycyclic hydrocarbons are located. When particulate matter adheres to skin, penetration of these polycyclic hydrocarbons is possible to a higher degree. Skin metabolizes these harmful molecules by benzo[a]pyrene hydroxylase. Increases in the amount and duration of exposure to polycyclic hydrocarbons on skin led to adjustment of the benzo[a]pyrene hydroxylase level [37].

It is evident that enzyme levels are modified not only by environmental molecules such as polycyclic hydrocarbons, but also by purposely applied compounds such as drugs in polymedicated patients. It is therefore advisable to also check drug interactions at the enzymatic level before prescription.

2.4 The Use of Animal Skin for the Prediction of Human Skin Metabolism

Excised human skin is rare, and the number of skin donators is often not sufficient for representative studies; therefore, animal skin is commonly used instead. The results obtained in this way are extrapolated to predict the penetration and permeation of drugs in human skin. Rittirod et al. [38] compared the permeation and metabolism of ethyl nicotinate (EN) to nicotinic acid (NA) in the skin of humans, rats, hairless rats, mice and hairless mice (Fig. 7).

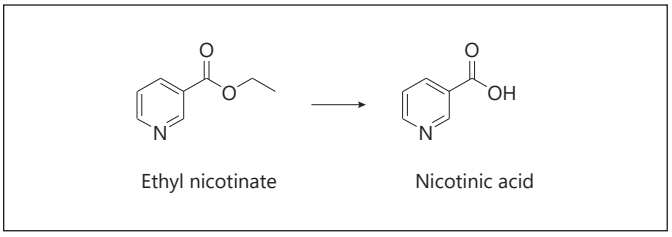

Fig. 7. Cleavage of the prodrug ethyl nicotinate to the parent drug nicotinic acid.

Clear differences were found between the tested species, due to the varying esterase activity. In human skin, an EN flux of 6.9 μmol/cm^2/h and an NA flux of 1.6 μmol/cm^2/h were measured. This corresponded most closely to the values for murine skin with an EN flux of 5.3 μmol/cm^2/h and an NA flux of 1.6 μmol/cm^2/h. In contrast, the greatest difference in metabolism was found in rat skin, with an EN flux of 0.4 μmol/cm^2/h and an NA flux of 6.0 μmol/cm^2/h. The ratio of EN to NA remarkably decreases from 4.3 for human skin to 0.07 for rat skin. Therefore, Rittirod et al. [38] suggested considering the species-dependent skin enzyme activity when predicting the skin permeability for human skin from an animal model.

3 Discussion

3.1 How Can Skin Metabolism Be Used for Dermal Drug Design?

3.1.1 The Concept of the Prodrug

Prodrugs are pharmacologically inactive derivates of parent drugs. After entering the viable epidermis, enzymes biotransform prodrugs into the respective pharmacologically active parent drugs. Prodrugs are formed when parent drugs possess unfavourable physicochemical properties for skin penetration. The final aim of the use of a prodrug always is an improvement of the parent drug's bioavailability. Until now, only dermal products have been formed that release the parent drug after hydrolytic cleavage by phase I esterases. In the following, a few examples of prodrugs are given.

3.1.1.1 Ethyl Nicotinate → Nicotinic Acid. Metabolism of EN to NA was already presented in Section 2.4 to better understand the species-dependent drug penetration and metabolism. NA is too hydrophilic to efficiently penetrate the SC. By ethylating the alcoholic group of NA, lipophilicity distinctly increases. The log*P* value increases from 0.36 to 1.17 and water solubility decreases from 18 to 0.05

g/L for NA and EN, respectively [39, 40]. This example illustrates well how strongly the masking of a hydrophilic alcohol group affects the physicochemical properties of a drug.

3.1.1.2 N-Acyl Derivate → 5-Fluorouracil. 5-Fluorouracil (5-FU) is a medication for the treatment of cancer. Dermally, 5-FU is applied for the treatment of actinic keratosis and basal cell carcinoma. Due to the high hydrophilicity of 5-FU, the principle of formulating a more lipophilic prodrug was applied. Nevertheless, this example differs, as a nitrogen group was masked instead of an alcohol group. Beall and Sloan [41] worked on two series of prodrugs: the alkyloxycarbonyl and the alkylcarbonyl series. Within each group, the prodrugs differed in the carbon chain length added to the parent drug. The rate of total delivered 5-FU through hairless murine skin was measured. All prodrugs delivered the parent drug more efficiently through skin, but the type of prodrug strongly affected the delivered amount of 5-FU: use of the alkylcarbonyl series resulted in far higher amounts of 5-FU. In addition, the carbon chain length played an important role: more 5-FU was detected with shorter carbon chains. For example, the 5-FU concentration measured was 40 times higher with C1, 20 times higher with C2 and 4 times higher with C6.

3.1.1.3 N,N′-Bis-Acyl Derivate → 5-Fluorouracil. In a second step, Beall and Sloan [42] masked both nitrogen groups of 5-FU. Based on the results of the preliminary investigations, only the 1,3-bis-alkylcarbonyl prodrugs were investigated, and the carbon chain lengths from C1 to C4 were used. Also for the 1,3-bis-alkylcarbonyl prodrugs, the highest delivery of 5-FU was detected with C1: the 5-FU concentration increased 5-fold. Thus, masking of both nitrogens was not beneficial when compared to the 40-fold increase in 5-FU delivery with the C1 N-alkylcarbonyl prodrug. What remained the same was the decrease in the delivered 5-FU amount with the increasing length of the carbon chain. Altogether, Beall and Sloan [42] did not recommend masking both hydrophilic groups when designing a prodrug of 5-FU.

3.1.1.4 Prednicarbate → 17- and 21-Ethyl Carbonate → Prednisolone. Prednicarbate (PC) can be used as a counterexample to the 1,3-bis-alkylcarbonyl prodrugs of 5-FU. This doubly masked prodrug can be sufficiently biotransformed by skin enzymes. Via two possible intermediates (17- and 21-ethyl carbonate), the parent drug PS is released in skin (Fig. 8). In excised human skin, esteratic OC cleavage proved dominant in the keratinocytes of viable skin [21]. After 6 h of exposure, 12% PS had permeated the skin. After 24 h of exposure, almost 83% of the applied PC was biotransformed. In tissue, the parent drug PS had the strongest presence, followed by 17-ethyl carbonate. Both PC and 21-ethyl carbonate were detected to a minor extent. In medium, only the parent drug PS was predominant. The amounts of 17- and 21-ethyl carbonate were comparable. The prodrug had by far the lowest presence. These values prove that skin enzymes can sufficiently biotransform both masked alcohol groups of the prodrug PC. Why PS is completely hydrolysed can possibly be explained by the special spatial structure of PS. The androstane skeleton may be considered a plane, and the side chains rise almost vertically out of this plane. The side chains are arranged in the opposite direction. The side chain on position 17 is directed down and that on position 21 is directed up; thus, both are sterically freely available to enzymes on each side.

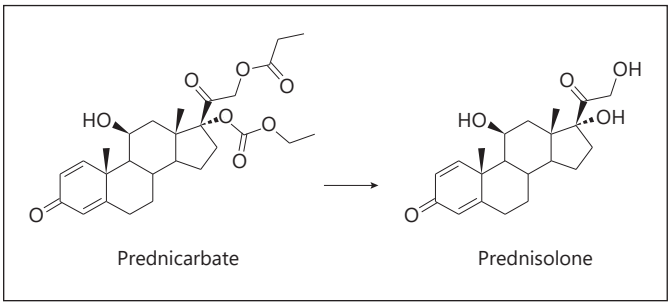

Fig. 8. Cleavage of the prodrug prednicarbate via two possible intermediates (17- and 21-ethyl carbonate) to the parent drug prednisolone.

3.1.2 The Concept of the Soft Drug

The use of soft drugs is a novel approach to the design of safer drugs for local skin therapy. In contrast to prodrugs, soft drugs are per se active. In the viable epidermis and dermis, skin enzymes biotransform them into inactive metabolites. The active drug, therefore, does not enter the systemic circulation and the pharmacological effect is limited to the application area. While designing soft drugs, considerations regarding their biotransformation are integrated. This rational design of rapidly and predictably inactivated soft drugs can be used conversely to create drugs resisting biotransformation via skin enzymes.

3.1.2.1 Benzoyl Peroxide → Benzoic Acid (Fig. 9). Benzoyl peroxide (BPO) was dermally applied to 5 patients for the treatment of leg ulcers [43]. Instead of BPO, only benzoic acid was detected in the patients' serum. These findings show that BPO is completely metabolized in the skin. Therefore, Morsches and Holzmann [43] excluded

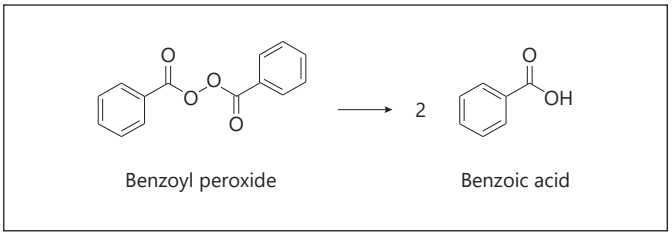

Fig. 9. Cleavage of the active drug benzoyl peroxide to inactive benzoic acid.

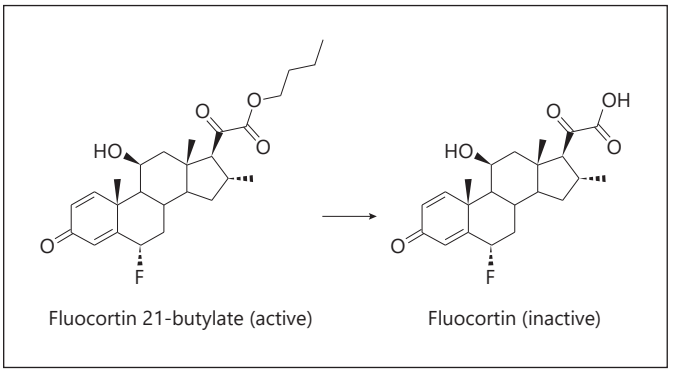

Fig. 10. Cleavage of the active drug fluocortin 21-butylate to inactive fluocortin.

a systemically toxic effect of BPO in local therapy. BPO proved to have a strong affinity for enzymatic cleavage. One explanation for this could be that peroxides have an electron-enriched molecule section. This may make them more vulnerable to enzymatic attacks. Consequently, electron-rich or -poor domains should be avoided when designing metabolism-resistant drugs.

3.1.2.2 Fluocortin 21-Butylate → Fluocortin (Fig. 10). Fluocortin 21-butylate, a synthetic glucocorticoid corticosteroid, is marketed in European countries for the treatment of skin diseases such as dermatosis, dermatitis, eczema, erythema, first-degree burns and insect bites. In analogy to PC, its androstane skeleton is arranged in a plane. The butyl ester is almost perpendicular to the plane, and therefore freely accessible for the skin's enzymes. The example of fluocortin 21-butylate demonstrates well that stereochemistry plays an important role in enzymatic degradation. For the design of TTS drugs, possible hydrolysable molecular sections should be arranged less sterically accessible.

3.1.3 Is There Any Stereoselective Biotransformation?

3.1.3.1 Racemic Ketoprofen Ethyl Ester → (R)- and (S)-Ketoprofen. Studies performed on HaCaT cells delivered results comparable to those with excised human skin regarding their biotransformation activity [21]. Ketoprofen is an NSAID used against pain and inflammation. (R)-Ketoprofen has mostly analgesic properties, whereas (S)-ketoprofen has 100- to 1,000-fold stronger anti-inflammatory characteristics. When orally administered, gastrointestinal side effects have frequently been reported, which is why transdermal application of ketoprofen has become more interesting. However, ketoprofen has physiochemical properties that are unsuitable for the transdermal route. Ketoprofen was formulated as a more lipophilic prodrug, ketoprofen ethyl ester (KEE). Both penetration and metabolism were investigated in HaCaT cells. The results showed that CE2 is mainly responsible for the biotransformation of KEE into ketoprofen. Suppression of CE2 selectively inhibited the hydrolysis of KEE almost completely [44] (Fig. 11). Although racemic KEE was applied to the HaCaT cells, almost exclusively the R-KEE was transformed into (R)-ketoprofen. For a dermal product with R/S-KEE as a prodrug, an analgesic but no anti-inflammatory effect can be expected.

3.1.3.2 Diastereomeric Propranolol Ester → Propranolol (Fig. 12). Propranolol is an effective β-blocker. (S)-Propranolol is 100 times more potent than (R)-propranolol. Nonetheless, due to the distinct hepatic first-pass metabolism of propranolol, its oral bioavailability is poor. For dermal delivery, propranolol is too hydrophilic. Thus, Udata et al. [45] synthesized a more lipophilic ester prodrug. Due to 2 chirality centres in the prodrug's molecule, 4 enantiomers exist (1S2S, 1S2R, 1R2R and 1R2S). Depending on their stereochemistry, their metabolism and flux into skin was evaluated. No stereospecific permeation in human skin was observed for racemic propranolol; both (S)- and (R)-propranolol permeated skin equally. In human skin tissue homogenates, the hydrolysis rates of the 4 enantiomers were different: 1S2R > 1R2R > 1R2S > 1S2S. These results are well in agreement with other investigations; here too, hydrolysis was more pronounced with the 2R enantiomers. An important new finding was that the flux rates were different between the 4 enantiomers; this shows the dependence of skin penetration on a drug's stereochemistry. In this study, the highest flux was obtained with the 1S2S enantiomer, being 30-fold higher than that of the parent drug. On the other hand, this enantiomer is metabolized most weakly to the parent drug. The question remains as to which of the 4 enantiomers provides the highest (S)-propranolol concentration in the blood in terms of both flux and metabolism.

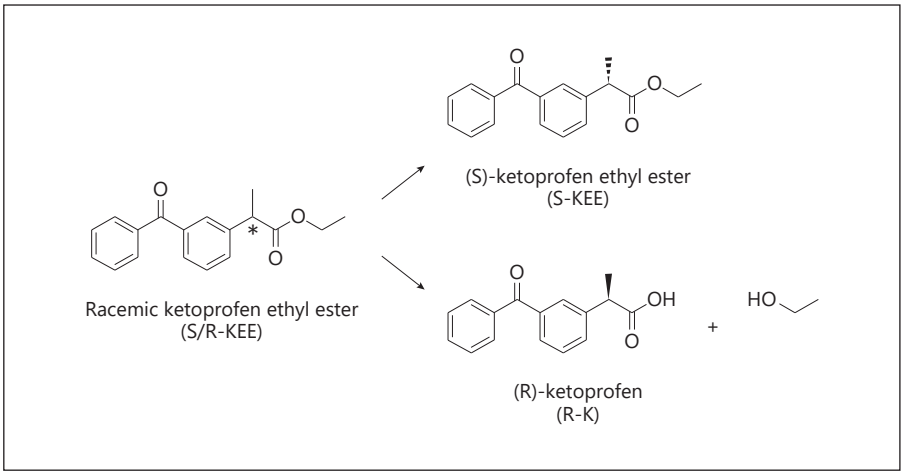

Fig. 11. Stereoselective cleavage of racemic ketoprofen ethyl ester primarily to (R)-ketoprofen.

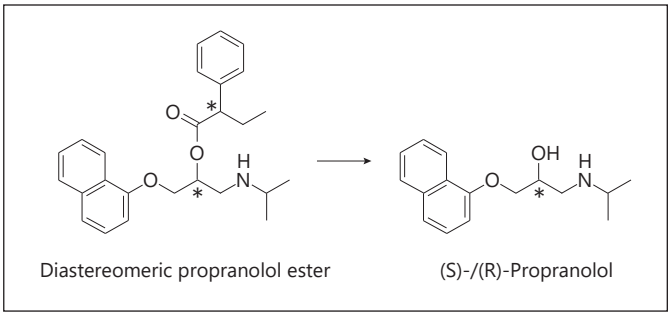

Fig. 12. Cleavage of diastereomeric propranolol ester to "monostereomeric" propranolol.

4 Conclusions

There are few recent publications that investigate skin enzymes' metabolizing activities affecting drug penetration and drug uptake. Since the types and activities of these enzymes are not comparable to those in other organs (e.g., the liver), more skin-specific research in this area is needed in order to draw any clear and firm conclusions. In addition, the fact that some metabolic processes are described in a controversial fashion makes it even more difficult to draw conclusions. However, we found enough similarities in these publications to allow us to make the following general recommendations. These recommendations will be useful for designing dermal drugs, since they pay attention to the issue of skin metabolism.

1. The types of skin enzyme vary with anatomical site and gender. Therefore, intra- and interindividual fluctuations can occur, especially if the drug is extensively metabolized.
2. Skin enzyme activity depends on age, becoming higher with increasing age. Thus, when using highly metabolized drugs in children, their doses should not only be reduced based on the child's weight.
3. By far the highest enzyme activity has been shown for the viable epidermis. Therefore, it is possible to effectively use both dermal prodrugs and dermal soft drugs, since both become activated or inactivated before entering the dermis, and thus before their systemic uptake.
4. The geometric properties of chiral drugs are maintained after their biotransformation. This allows utilizing the more active enantiomer when formulating prodrugs. If both enantiomers (R and S) are equally active, the R enantiomer can be used if high biotransformation is required (e.g., prodrugs and soft drugs), and the S enantiomer if the applied drug should be degraded to a lesser extent by the enzymes of the skin.
5. When using these prodrugs and soft drugs, thought should be given to enzyme activity saturation. Esterification of more than one hydroxyl group (to obtain a more lipophilic prodrug) often leads to a lower release of the parent drug because the sterically more accessible esters or ethers are cleaved first.
6. Instead of masking several hydroxyl groups, the carbon chain length may be varied to increase lipophilicity. In most cases, enzymatic biotransformation was enhanced with increasing carbon chain lengths. In addition, aliphatic groups should not be branched if high biotransformation is desired. Here again, steric hindrance seems to play an important role in skin metabolism.

Acknowledgements

Sung Min Pyo gratefully acknowledges financial support from the women's promotion of the Department of Biology, Chemistry, and Pharmacy, Freie Universität Berlin.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

References

1 Front Line Strategic Consulting Inc. Alternative Drug Delivery Systems Series: Transdermal Drug Delivery Systems. Front Line Strategic Consulting Inc.; 2002.
2 Prescient & Strategic Intelligence [Internet]. New York: Transdermal Drug Delivery Systems Market Overview [cited 2019 Feb 22]. Available from: https://www.psmarketresearch.com/market-analysis/transdermal-drug-delivery-systems-market.
3 Research Nester [Internet]. Global Transdermal Drug Delivery Market Analysis Opportunity Outlook 2024 [cited 2019 Feb 22]. Available from: https://www.researchnester.com/reports/global-transdermal-drug-delivery-market-analysis-opportunity-outlook-2024/111.
4 Murphy M, Carmichael AJ. Transdermal drug delivery systems and skin sensitivity reactions. Incidence and management. Am J Clin Dermatol. 2000 Nov-Dec;1(6):361–8.
5 Elias PM, Menon GK. Structural and lipid biochemical correlates of the epidermal permeability barrier. Adv Lipid Res. 1991;24(6):1–26.
6 Ranade VV. Drug delivery systems. 6. Transdermal drug delivery. J Clin Pharmacol. 1991 May;31(5):401–18.
7 Martinović S, Pasa-Tolíc L, Masselon C, Jensen PK, Stone CL, Smith RD. Characterization of human alcohol dehydrogenase isoenzymes by capillary isoelectric focusing-mass spectrometry. Electrophoresis. 2000 Jul;21(12):2368–75.
8 Cheung C, Smith CK, Hoog JO, Hotchkiss SA. Expression and localization of human alcohol and aldehyde dehydrogenase enzymes in skin. Biochem Biophys Res Commun. 1999 Jul;261(1):100–7.
9 Wilkin JK, Stewart JH. Substrate specificity of human cutaneous alcohol dehydrogenase and erythema provoked by lower aliphatic alcohols. J Invest Dermatol. 1987 Apr;88(4):452–4.
10 Oesch F, Fabian E, Oesch-Bartlomowicz B, Werner C, Landsiedel R. Drug-metabolizing enzymes in the skin of man, rat, and pig. Drug Metab Rev. 2007;39(4):659–98.
11 Janmohamed A, Dolphin CT, Phillips IR, Shephard EA. Quantification and cellular localization of expression in human skin of genes encoding flavin-containing monooxygenases and cytochromes P450. Biochem Pharmacol. 2001 Sep;62(6):777–86.
12 Hu T, Khambatta ZS, Hayden PJ, Bolmarcich J, Binder RL, Robinson MK, et al. Xenobiotic metabolism gene expression in the EpiDermin vitro 3D human epidermis model compared to human skin. Toxicol In Vitro. 2010 Aug;24(5):1450–63.
13 Jäckh C, Blatz V, Fabian E, Guth K, van Ravenzwaay B, Reisinger K, et al. Characterization of enzyme activities of Cytochrome P450 enzymes, flavin-dependent monooxygenases, N-acetyltransferases and UDP-glucuronyltransferases in human reconstructed epidermis and full-thickness skin models. Toxicol In Vitro. 2011 Sep;25(6):1209–14.
14 Vyas PM, Roychowdhury S, Koukouritaki SB, Hines RN, Krueger SK, Williams DE, et al. Enzyme-mediated protein haptenation of dapsone and sulfamethoxazole in human keratinocytes: II. Expression and role of flavin-containing monooxygenases and peroxidases. J Pharmacol Exp Ther. 2006 Oct;319(1):497–505.
15 Venkatesh K, Levi PE, Inman AO, Monteiro-Riviere NA, Misra R, Hodgson E. Enzymatic and immunohistochemical studies on the role of cytochrome P450 and the flavin-containing monooxygenase of mouse skin in the metabolism of pesticides and other xenobiotics. Pestic Biochem Physiol. 1992;43(1):53–66.
16 Yoshida A, Rzhetsky A, Hsu LC, Chang C. Human aldehyde dehydrogenase gene family. Eur J Biochem. 1998 Feb;251(3):549–57.
17 Hosokawa M, Furihata T, Yaginuma Y, Yamamoto N, Koyano N, Fujii A, et al. Genomic structure and transcriptional regulation of the rat, mouse, and human carboxylesterase genes. Drug Metab Rev. 2007;39(1):1–15.
18 McCracken NW, Blain PG, Williams FM. Nature and role of xenobiotic metabolizing esterases in rat liver, lung, skin and blood. Biochem Pharmacol. 1993 Jan;45(1):31–6.
19 Sogorb MA, Vilanova E. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicol Lett. 2002 Mar;128(1-3):215–28.
20 Gysler A, Kleuser B, Sippl W, Lange K, Korting HC, Höltje HD, et al. Skin penetration and metabolism of topical glucocorticoids in reconstructed epidermis and in excised human skin. Pharm Res. 1999 Sep;16(9):1386–91.
21 Bätz FM, Klipper W, Korting HC, Henkler F, Landsiedel R, Luch A, et al. Esterase activity in excised and reconstructed human skin – biotransformation of prednicarbate and the model dye fluorescein diacetate. Eur J Pharm Biopharm. 2013 Jun;84(2):374–85.
22 Prusakiewicz JJ, Ackermann C, Voorman R. Comparison of skin esterase activities from different species. Pharm Res. 2006 Jul;23(7):1517–24.
23 Barker CL, Clothier RH. Human keratinocyte cultures as models of cutaneous esterase activity. Toxicol In Vitro. 1997 Oct;11(5):637–40.
24 Lereaux G, Eilstein J, Meunier JH, Leclaire J, Duche D. Characterization of Esterase, Glucuronyl and Sulfo Transferase Activities in Skin and Reconstructed Human Skin Models. Drug Metab Rev. 2010;42(8):149–50.
25 Hayden P, Bolmarcich J, Stolper G, Hu T, Aardema M, Curren R, et al. Xenobiotic metabolizing capabilities of the EpiDerm in vitro human skin equivalent: utility for assessing dermal biotransformation of pharmaceuticals and environmental chemicals. Toxicol Lett. 2006;164(7):S225–6.
26 Hikima T, Maibach HI. Distribution of hydrolytic activity catalyzes the biotransformation of prednisolone 21-acetate in human skin. Skin Pharmacol Appl Skin Physiol. 2001 Jul-Aug;14(4):196–202.
27 Hatanaka T. Skin Metabolism of Chemicals. In: Sugibayashi K, editor. Skin Permeation and Disposition of Therapeutic and Cosmeceutical Compounds. Tokyo: Springer Japan; 2017. pp. 67–76.
28 Stüttgen G, Schaefer H. Der Stoffwechsel der Haut. In: Stüttgen G, Schaefer H, editors. Funktionelle Dermatologie. Volume 1. Springer; 1974. pp. 42–59.
29 Ngawhirunpat T, Kawakami N, Hatanaka T, Kawakami J, Adachi I. Age dependency of esterase activity in rat and human keratinocytes. Biol Pharm Bull. 2003 Sep;26(9):1311–4.
30 Hsia SL, Hao YL. Metabolic transformations of cortisol-4-[14C] in human skin. Biochemistry. 1966 May;5(5):1469–74.
31 Onoue S, Kobayashi T, Takemoto Y, Sasaki I, Shinkai H. Induction of matrix metalloproteinase-9 secretion from human keratinocytes in culture by ultraviolet B irradiation. J Dermatol Sci. 2003 Nov;33(2):105–11.

32 Katiyar SK, Matsui MS, Mukhtar H. Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. J Invest Dermatol. 2000 Feb;114(2):328–33.
33 Kulms D, Schwarz T. Molecular mechanisms of UV-induced apoptosis. Photodermatol Photoimmunol Photomed. 2000 Oct;16(5):195–201.
34 Obermüller-Jevic UC, Schlegel B, Flaccus A, Biesalski HK. The effect of β-carotene on the expression of interleukin-6 and heme oxygenase-1 in UV-irradiated human skin fibroblasts in vitro. FEBS Lett. 2001 Dec;509(2):186–90.
35 Allanson M, Reeve VE. Ultraviolet A (320–400 nm) modulation of ultraviolet B (290–320 nm)-induced immune suppression is mediated by carbon monoxide. J Invest Dermatol. 2005 Mar;124(3):644–50.
36 Goldsmith PC, Leslie TA, Hayes NA, Levell NJ, Dowd PM, Foreman JC. Inhibitors of nitric oxide synthase in human skin. J Invest Dermatol. 1996 Jan;106(1):113–8.
37 Alvares AP, Kappas A, Levin W, Conney AH. Inducibility of benzo[α]pyrene hydroxylase in human skin by polycylic hydrocarbons. Clin Pharmacol Ther. 1973 Jan-Feb;14(1):30–40.
38 Rittirod T, Hatanaka T, Uraki A, Hino K, Katayama K, Koizumi T. Species difference in simultaneous transport and metabolism of ethyl nicotinate in skin. Int J Pharm. 1999 Feb;178(2):161–9.
39 Sangster J. LOGKOW A Databank of Evaluated Octanol-Water Partition Coefficients (Log P) on Microcomputer Diskette. Sangster Research Laboratories Canadian National Committee for CODATA; 1993.
40 Yalkowsky SH, He Y, Jain P. Handbook of aqueous solubility data. CRC Press; 2016.
41 Beall HD, Sloan KB. Transdermal delivery of 5-fluorouracil (5-FU) by 1-alkylcarbonyl-5-FU prodrugs. Int J Pharm. 1996;129(1-2):203–10.
42 Beall HD, Sloan KB. Topical delivery of 5-fluorouracil (5-FU) by 1,3-bisalkylcarbonyl-5-FU prodrugs. Int J Pharm. 2002 Jan;231(1):43–9.
43 Morsches B, Holzmann H. [Studies on the percutaneous absorption of benzoyl peroxide (author's transl)]. Arzneimittelforschung. 1982;32(3):298–300.
44 Zhu QG, Hu JH, Liu JY, Lu SW, Liu YX, Wang J. Stereoselective characteristics and mechanisms of epidermal carboxylesterase metabolism observed in HaCaT keratinocytes. Biol Pharm Bull. 2007 Mar;30(3):532–6.
45 Udata C, Tirucherai G, Mitra AK. Synthesis, stereoselective enzymatic hydrolysis, and skin permeation of diastereomeric propranolol ester prodrugs. J Pharm Sci. 1999 May;88(5):544–50.