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Review article

Bioavailability and bioequivalence of topical glucocorticoids

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Abstract

Dermatological therapy depends significantly upon the use of topical glucocorticoids (TG). While effective, and often remarkably so, their delivery into the skin is sometimes rather inefficient. The factors which determine drug efficacy, and the various methodologies, by which the rate and extent of TG transport to their sites of action may be assessed, are the foci of this review.

Keywords: Topical glucocorticoids; Bioavailability; Bioequivalence; Percutaneous absorption; Side-effects; Vehicle effects; Potency

1. Introduction

Topical glucocorticoids (TG) are the most frequently prescribed drugs by dermatologists. Their clinical effectiveness in the treatment of psoriasis and atopic dermatitis is related to their vasoconstrictive, anti-inflammatory, immunosuppressive and anti-proliferative effects. Treatment with TG formulations is effective, easy to administer, acceptable to patients and safe when used correctly. Since their introduction in the early 1950s they have revolutionized treatment of inflammatory skin disease. This review focuses on the main factors influencing the effectiveness and bioavailability of TG namely (i) the structure of the skin barrier, (ii) effects and side-effects of TG, (iii) chemical modifications in the TG structure, (iv) vehicle or formulation effects, and (v) methods to determine bioavailability and/or bioequivalence (BA/BE).

2. The skin barrier

The skin is the largest organ of the body with an area of approximately 2 m^2 and is the interface between the organism and its environment. It prevents the loss of water and the ingress of foreign materials. In essence, the skin consists of three functional layers: the epidermis, the dermis (corium) and the hypodermis. The hypodermis is a subcutaneous tissue consisting of fat and muscle and acts as a heat isolator, a shock absorber, and an energy storage region. The dermis is ~2 mm thick and contains collagen, elastic fibers, blood vessels, nerves as well as hair follicles and sebaceous and sweat glands. The main cells in the dermis are fibroblasts, which are involved in the immune and inflammatory response and upon which glucocorticoid receptors are found. The dermis is the source of nutrients for the epidermis. Because the epidermis is avascular, essential substances are transported only by passive diffusion. The epidermis has a multilayered structure reflecting different stages of differentiation of the skin cells (the keratinocytes). From the proliferative, basal layer, the cells change in an ordered fashion from metabolically active and dividing to dense, functionally dead, and fully keratinized, the so-called corneocytes. These corneocytes are embedded in a lipid matrix and form the outer 10–20 μm of the epidermis, the stratum corneum (SC) [1]. In this lipid matrix, however, different enzymes (proteases) were found which are involved in the desquamation process [2].

The cytoplasm of cultured human skin keratinocytes and fibroblasts contains macromolecules that bind glucocorticoids with high affinity, suggesting that the site of

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action for TG are both epidermal and dermal cells [3,4]. To reach these target cells, TG have to permeate the SC (Fig. 1), which contributes the major barrier to percutaneous absorption [5,6].

The terminally differentiated corneocytes (bricks) consist primarily of a highly organized keratin microfibrillar matrix, which provides mechanical resistance. Natural moisturizing factor (NMF), a mixture of amino acids, lactic acid, urea, citrate and sugar, is present at a high level in the corneocytes and acts as a very efficient humectant [8]. In addition, protein junctions, the corneodesmosomes, link adjacent corneocytes and ensure the cohesiveness of this layer [9–11]. During the formation and maturation of the SC, desmosomes are modified and their number decreases towards the skin surface [12,13]. Each corneocyte is surrounded by a 15–20 nm thick protein shell – the cornified cell envelope (CE), a 15 nm layer of defined structural proteins and a 5 nm thick layer of specialized lipids. The lipid monolayer provides a hydrophobic interface between the CE itself and SC lipid lamellae and helps maintain water barrier function [14,15]. While typical biological membranes are mainly composed of phospholipids, the intercellular SC lipids (mortar) comprise primarily ceramides (~40% w/w), free fatty acids (~10% w/w) and cholesterol (~25% w/w), together with a small fraction of cholesterol sulphate and triglycerides (Fig. 2) [16–19]. The lipids originate from lamellar bodies that are synthesized in the upper viable layers of the epidermis, which are ultimately secreted from the cells into the intercellular space. These lipids, which are organized in multilamellar bilayers, regulate the passive flux of water through the SC and are considered to be very important for skin barrier function [20–25].

Nine classes of ceramides have been recognized and differ from each other by the head group architecture and by the average fatty acid chain length [26,27]. Ceramides 1 and 9 are believed to be particularly important [28–30]. A direct relationship between the degree of barrier perturbation (measured as transepidermal water loss) and the amount of SC lipid removed has been demonstrated [31].

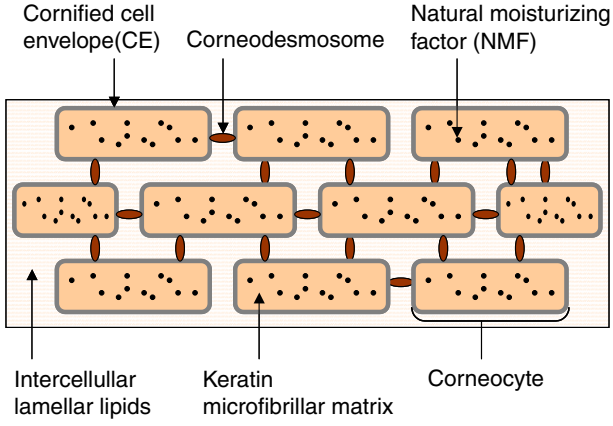

Fig. 1. Schematic 'brick and mortar' representation of the structural and functional components of the SC. Modified from Harding [7].

This 'brick and mortar' arrangement of the SC creates a tortuous route for compounds to permeate the barrier.

3. Percutaneous absorption and mechanism of TG action

The percutaneous absorption of a drug from a topically applied formulation is a complex process [32,33]. The physicochemical characteristics of the drug and the vehicle and the physiological conditions of the skin can significantly affect percutaneous absorption. For a topically applied formulation containing a glucocorticoid the percutaneous absorption involves the following steps:

(a) release from the formulation,
(b) penetration into the skin's outermost layer, the SC and permeation/diffusion through the SC,
(c) partitioning from the SC into the viable epidermis and the dermis,
(d) within the viable epidermis/dermis, diffusion to reach the glucocorticoid receptor and binding.

Penetration into the SC is assumed to be the rate-limiting step for percutaneous absorption of TG. In this case, the glucocorticoid amount reaching the target cell will be determined by its partitioning into and rate of transport through the SC. These processes are greatly influenced by the physicochemical properties of both the drug and the vehicle [34,35]. The *in vivo* clinical effectiveness of a TG formulation depends on the bioavailability of the glucocorticoid within the skin at the site of action. For TG the target cells are the keratinocytes and fibroblasts within the viable epidermis and dermis, where the glucocorticoid receptors are located [3,36]. Having attained the target, the cellular uptake and residence time of the steroid as well as its affinity for the glucocorticoid receptor will determine the clinical effect [4,37–39]. From studies with cultured human fibroblasts and keratinocytes, the cellular uptake of glucocorticoids has been shown to be a non-mediated, passive diffusion process that involves two intrinsic steps: a rapid, non-specific, high-capacity association to the cell membrane followed by a slower process leading to strong binding of glucocorticoid within the cell [38]. The total uptake of steroid by fibroblasts and keratinocytes was related to drug lipophilicity. Although, as stated, steroids are generally thought to be transported across the cell membrane by passive diffusion, there is some evidence that certain target cells possess a specific transport system for these compounds [40].

The anti-inflammatory and immunosuppressive effects of TG seem to be mediated largely by regulation of corticosteroid-responsive genes (Fig. 3). Within the cytoplasm, the steroid binds to the glucocorticoid receptor, forming a complex that is rapidly transported to the nucleus. The glucocorticoid-receptor complex inside the nucleus then binds to a region of DNA called the glucocorticoid responsive element (GRE) to either stimulate or inhibit transcription and regulate thereby the inflammatory process [41,42].

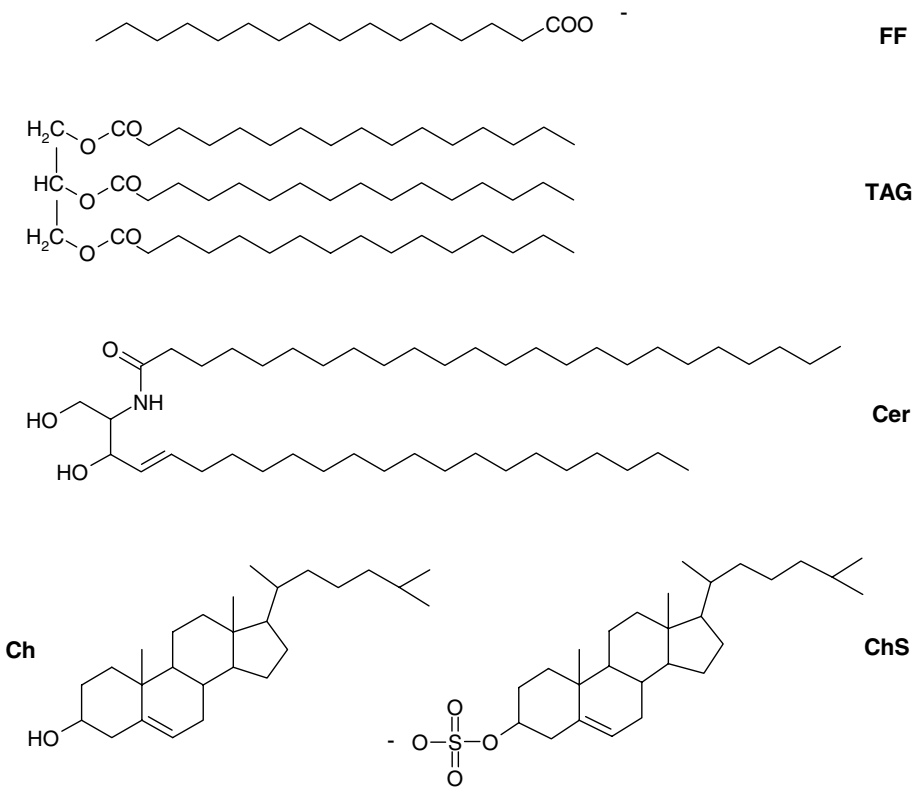

Fig. 2. Basic structures of SC lipids: free fatty acids (FF), triglycerides (TAG), ceramides (Cer), cholesterol (Ch), cholesterol sulphate (ChS).

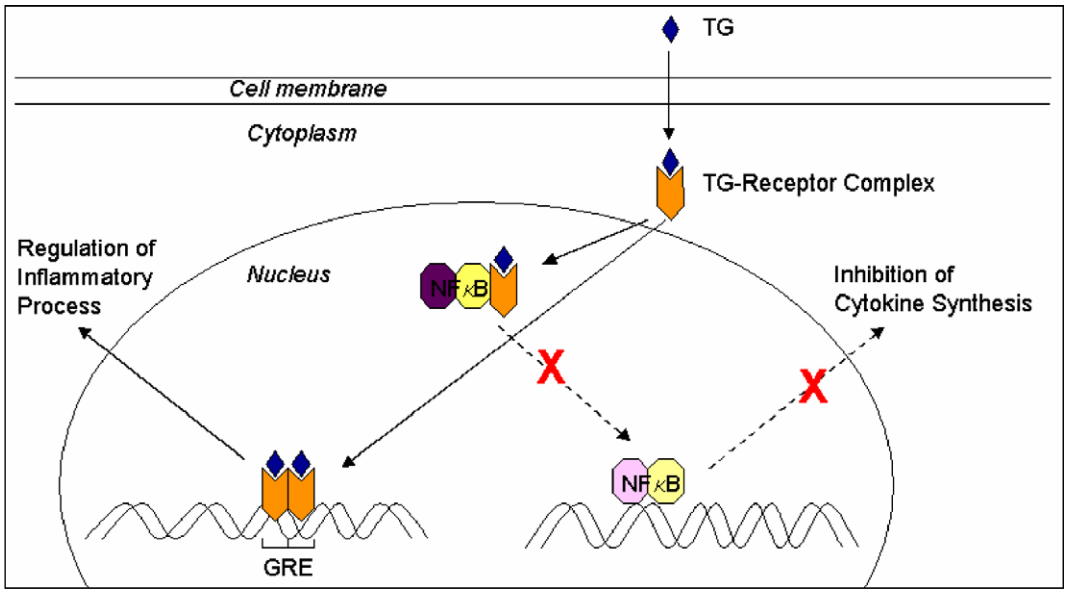

Fig. 3. Schematic representation of mechanism of action of topical glucocorticoids (TG). Nuclear factor-κB (NF-κB), Glucocorticoid response element (GRE). Modified from Norris [47].

In addition to this direct regulatory effect on gene transcription, TG are also able to indirectly regulate transcription by blocking the effects of other transcription factors, such as nuclear factor-κB [43,44]. TG may inhibit the transcription of proinflammatory cytokine genes (including the interleukins IL-1, IL-2, IL-6, interferon γ, and tumour necrosis factor-α genes), T-cell proliferation, and T-cell dependent immunity [45]. In fibroblasts, IL-1α is responsible for proliferation, collagenase induction, and IL-6 synthesis, which control skin thickness [46]. The inhibition of IL-1α in keratinocytes has anti-inflammatory effects, whereas the same inhibition in fibroblasts has anti-prolifer-

ative and atrophogenic effects. The vasoconstrictive effect of TG may contribute to their anti-inflammatory activity, diminishing erythema at the lesion site. It may also reduce their local clearance. However, the exact mechanism is not completely clear.

Finally, clinical efficacy is self-evidently and significantly influenced by corticosteroid structure, the formulation and to some extent the applied concentration of the drug [48–52]. Because dose applied is very loosely related to dose absorbed, the applied concentration is much less a factor than the formulation. These factors are described later in much greater detail.

4. Side-effects

Despite their clear benefit in the therapy of inflammatory disease, TG are associated with a number of side-effects that limit their use. One particularly important local side-effect is epidermal thinning or atrophy [53]. This effect can start after 3 to 14 days of corticosteroid treatment with microscopic degenerative changes in the epidermis, including reduction in cell size and number of cell layers [41,54]. TG inhibit epidermal cell differentiation by inhibition of keratinocyte proliferation and acceleration of keratinocyte maturation [55–57].

Moreover, prolonged TG therapy increases basal transepidermal water loss, indicating an effect on permeability barrier function [54]. This change has been associated with a decrease in SC thickness, a reduction in lipid content [54], a decrease in the number of lamellar bodies [56] and in the number of intercellular lamellae (although the structure of these lipid bilayers appeared normal [54]).

Even short-term treatment with a potent TG, clobetasol (0.05% w/V), applied once a day for 3 days can alter epidermal structure and function in humans [58]. SC barrier recovery was significantly delayed, even though visible changes in the epidermis were not observed [58]. In hairless mouse skin (the barrier function of which is, of course, less effective), the same short-term treatment inhibited epidermal cholesterol, fatty acid, and ceramide synthesis by more than 50%. This inhibition may account for the decreased production and secretion of lamellar bodies and the impaired formation of lipid bilayers in the SC and the resulting abnormality in barrier function [58]. TG also exerted negative effects on the integrity and cohesion of the SC owing to a reduction in the number of corneodesmosomes in the SC [58].

Furthermore, histological changes are observed in the dermis. Dermal atrophy results from the direct anti-proliferative action of TG on fibroblasts [59]; in turn, this leads to a reduction in the synthesis of collagen and mucopolysaccharides and a loss of dermal support. The elastin fibers in the upper layers of the dermis become thin and fragmented, while the deeper fibers collapse to form a compact and dense network. As a result of this thin and brittle skin, there is local vascular dilatation, which is responsible for striae, telangiectasia, and purpura [41,60]. These side-effects are seen mainly on the (permeable) face and are (over) dose related.

Systemic side-effects of TG, such as pituitary–adrenal axis suppression, are rare but have to be seriously considered when treating children because of the potential for growth retardation. Furthermore, children have a higher ratio of total body surface area to body weight (about 2.5- to 3-fold that of adults). The degree of adrenal suppression increases with the potency and concentration of the TG, application area, occlusion and degree of inflamed skin. Other systemic side-effects include Cushing's syndrome, the aggravation of diabetes mellitus, and increasing or causing hypertension and osteonecrosis.

5. Glucocorticoid chemistry

In humans, the naturally occurring corticosteroid is cortisol, or hydrocortisone, which is produced primarily in the adrenal gland. The majority of TG that are used therapeutically are synthetic derivatives of hydrocortisone (Fig. 4). Hydrocortisone has an androstane structure arranged in four rings, with a relative low potency and a short duration of action. The free hydroxyl group at C-11 is essential for the topical activity. The first ever trial on TG was on cortisone (the C-11 ketone) in 1948 which is active orally but is not effective topically. Luckily, science preserved and hydrocortisone was shown to be effective topically in 1950.

Decreased mineralocorticoid and increased glucocorticoid activity (increased affinity for the corticosteroid receptor) can be achieved by introduction of an additional double bond at C-1 and substitution at the C-16 position (Fig. 5). The lipophilicity of the steroid and the duration of action are greatly increased by fluorination of the B ring at the C-9 and/or C-6 position. Moreover the lipophilicity and metabolic resistance of TG may also be increased by adding ester or acetal groups to the D-ring (e.g., betamethasone 17-valerate). Clobetasol propionate is the most potent of the currently available TG.

Strategies to optimize the potency and, in particular, the anti-inflammatory and immunosuppressive capacity of TG, while minimizing their adverse effects have been pursued. But 'ideal' TG have not yet been synthesized. Prednicarbate, hydrocortisone aceponate, mometasone furoate and methylprednisolone aceponate are the first, so-called soft-steroids. These are 17,21-double esters of hydrocortisone with significant anti-inflammatory activity, but with the least capacity to induce skin atrophy [61–64]. The relative weak side-effects are the result of a specific metabolic step and a selective influence on cytokine production.

In vivo, mometasone furoate, methylprednisolone aceponate and hydrocortisone induced similar skin atrophy but the soft steroids elicited a much greater blanching response [65]. Similarly, prednicarbate caused significantly less skin atrophy than the equipotent betamethasone 17-valerate [66]. This low atrophogenic effect is the result of its minor effects on IL-1α and IL-6 suppression in dermal fibroblasts; the high degree of IL-1α suppression in epider-

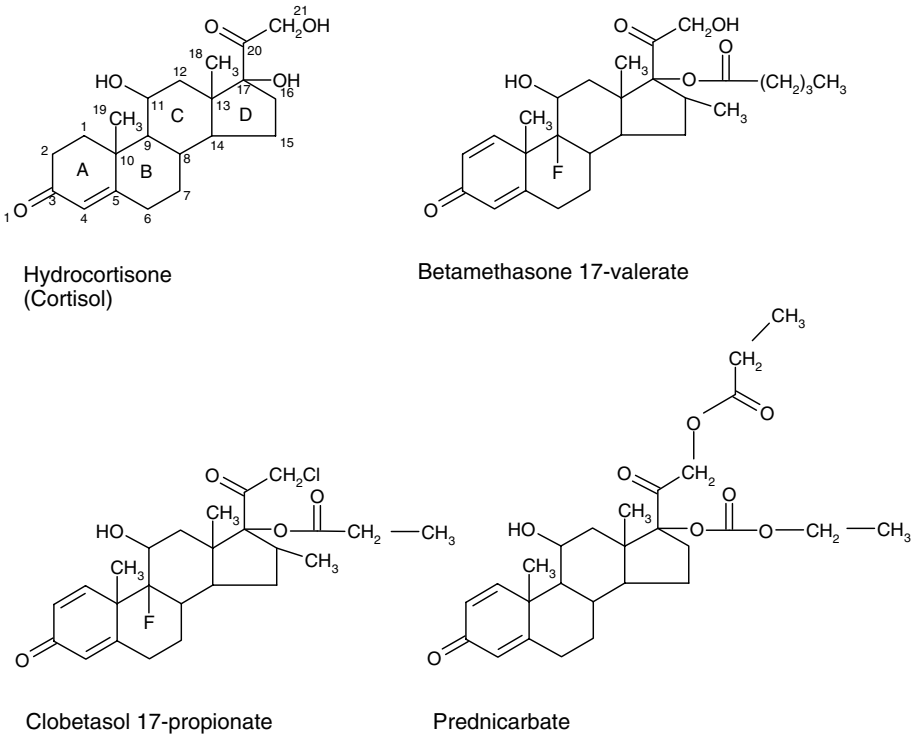

Fig. 4. Structure of hydrocortisone and selected TG of greater potency.

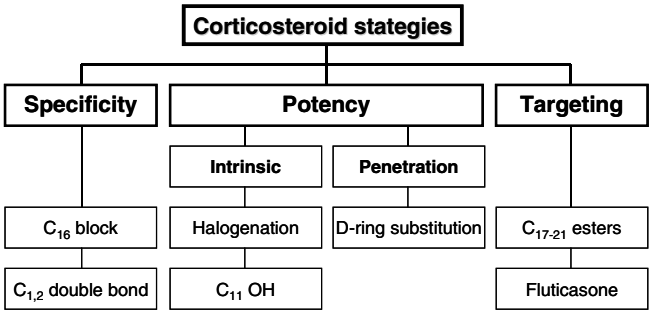

Fig. 5. Strategies to optimize TG activity.

mal keratinocytes, on the other hand, is almost equivalent to that of betamethasone 17-valerate [46,67,68]. Prednicarbate (PC) penetrates the epidermis more readily due to esterification at position 17 and 21. Reaching the keratinocytes, PC is rapidly metabolized to prednisolone 17-ethylcarbonate (PEC), which is believed to be primarily responsible for the anti-inflammatory effect due to its higher receptor affinity. In fibroblasts, PEC inhibits IL-1α and IL-6 much more than PC. However, the metabolism of PC in fibroblasts is only 1% per hour, possibly due to low esterase activity. Moreover, the permeation of PC through the epidermis into the dermis is very slow, resulting in a negligible atrophogenic effect [68,69]. Therefore, these compounds can be used to treat sensitive areas, such as the face, and large surface areas in children, with minimal local and systemic side-effects [62]. Fluticasone propionate (Fig. 6), a fluoromethyl androstane-17β-carbiothioate, is another new soft steroid with good anti-inflammatory activity but a much lower potential to cause systemic side-effects. The two esterifications at positions 17 and 20 increase the molecule's lipophilicity, and its uptake by and affinity for the glucocorticoid receptor. The small amount of drug that is systemically absorbed is rapidly metabolized to the inactive carboxylic acid derivative in the liver [70].

6. Potency/classification

The efficacy of a TG is related to its pharmacological potency and to its ability to be absorbed into the target cells within the viable epidermis and dermis [71]. Potency is a complex function of the physical and chemical properties of both the drug and its vehicle [50,72]. For the TG, a ranking of drugs and vehicles has been evolved using the skin blanching assay.

The American classification includes seven potency groups (Table 1) [73], while the British National Formulary recommends only four (Table 2) [74]. In the former system, the potency of a product is characterized by the corticosteroid, its concentration and the nature of the vehicle. Corticosteroid formulations in the same potency group have similar efficacy and a similar potential to provoke side-effects. That is, the greater the potency, the greater the therapeutic efficacy, but also the greater the adverse effects. Low-potency formulations are considered acceptable for long-term treatments while the more potent products should be reserved for shorter regimes and for use at sites, such as the palms and soles, where low potency corticoste-

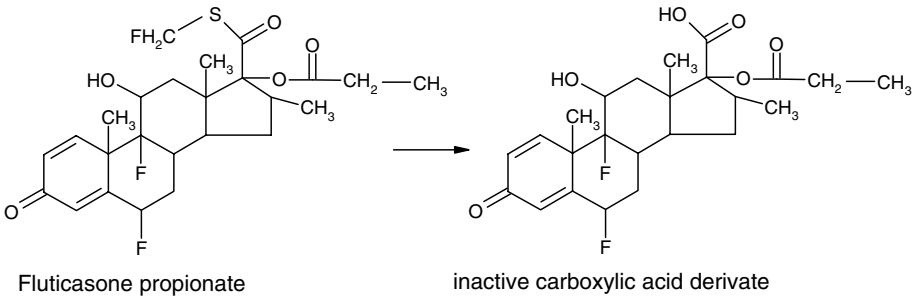

Fig. 6. Fluticasone propionate and its inactive metabolite.

roids are ineffective [75]. The British classification system is made irrespective of the topical vehicle used.

The dependence of the pharmacological response on the drug concentration in the vehicle is classically illustrated by a 'dose–response' curve (Fig. 7) [76]. The profile is characterized by (i) a threshold concentration S, which is the minimum necessary to induce a response, (ii) a range over which the response increases linearly with the logarithm of the 'dose', (iii) the EC_{50}, the concentration that elicits 50% of the maximal effect, and (iv) a plateau (E_{max}), where further increases in concentration provoke no additional pharmacological response. Clearly, different formulations of the same steroid can shift the position of the 'dose–response' curve by either enhancing or retarding the drug's penetration into the skin. The bioavailability factor f can be estimated from the horizontal distance between parallel curves.

As will be shown in the next section, vehicle effects tend to dominate those of dose.

7. Vehicles and formulations

Based on the Higuchi model, in the early 1970s, Katz and Poulsen described the significance of both release (diffusion out of the vehicle) and penetration (diffusion into the skin barrier) for topical product design based on corticosteroids [77]. These two processes are dependent upon the physico-chemical properties of both the drug and the vehicle [78].

Lippold and Schneemann showed that the skin blanching response of betamethasone 17-benzoate increased proportionally with the concentration of the dissolved and diffusible drug, reaching the maximal response when the concentration equals the solubility of the drug in the vehicle (Fig. 8) [79]. The blanching response from suspensions of the drug was independent of the betamethasone 17-benzoate concentration [79]. In Fig. 8, the blanching response of the test formulations (B_T) was related to the maximum blanching response of an internal standard (B_{ST}).

The amount of drug needed for the same blanching response differs according to the steroid solubility in the vehicle. However, the same thermodynamic activity of the drug in the vehicle leads to the same pharmacodynamic response, provided that the formulation does not change

Table 1
Classification of TG according to potency by the National Psoriasis Foundation (from www.psoriasis.org)

TG product	Incorporated TG
CLASS I – Superpotent	
Clobex Lotion, 0.05%	Clobetasol propionate
Cormax Cream/Solution, 0.05%	Clobetasol propionate
Diprolene Gel/Ointment, 0.05%	Betamethasone dipropionate
Olux Foam, 0.05%	Clobetasol propionate
Psorcon Ointment, 0.05%	Diflorasone diacetate
Temovate Cream/Ointment/Solution, 0.05%	Clobetasol propionate
Ultravate Cream/Ointment, 0.05%	Halobetasol propionate
CLASS II – Potent	
Cyclocort Ointment, 0.1%	Amcinonide
Diprolene Cream AF, 0.05%	Betamethasone dipropionate
Diprosone Ointment, 0.05%	Betamethasone dipropionate
Elocon Ointment, 0.1%	Mometasone furoate
Florone Ointment, 0.05%	Diflorasone diacetate
Halog Ointment/Cream, 0.1%	Halcinonide
Lidex Cream/Gel/Ointment, 0.05%	Fluocinonide
Maxiflor Ointment, 0.05%	Diflorasone diacetate
Maxivate Ointment, 0.05%	Betamethasone dipropionate
Psorcon Cream, 0.05%	Diflorasone diacetate
Topicort Cream/Ointment, 0.25%	Desoximetasone
Topicort Gel, 0.05%	Desoximetasone
CLASS III – Upper Mid-Strength	
Aristocort A Ointment, 0.1%	Triamcinolone acetonide
Cutivate Ointment, 0.005%	Fluticasone propionate
Cyclocort Cream/Lotion, 0.1%	Amcinonide
Diprosone Cream, 0.05%	Betamethasone dipropionate
Florone Cream, 0.05%	Diflorasone diacetate
Lidex-E Cream, 0.05%	Fluocinonide
Luxiq Foam, 0.12%	Betamethasone valerate
Maxiflor Cream, 0.05%	Diflorasone diacetate
Maxivate Cream/Lotion, 0.05%	Betamethasone dipropionate
Topicort Cream, 0.05%	Desoximetasone
Valisone Ointment, 0.1%	Betamethasone valerate
CLASS IV – Mid-Strength	
Aristocort Cream, 0.1%	Triamcinolone acetonide
Cordran Ointment, 0.05%	Flurandrenolide
Derma-Smoothe/FS Oil, 0.01%	Fluocinolone acetonide
Elocon Cream, 0.1%	Mometasone furoate
Kenalog Cream/Ointment/Spray, 0.1%	Triamcinolone acetonide

Table 1 (*continued*)

TG product	Incorporated TG
Synalar Ointment, 0.025%	Fluocinolone acetonide
Uticort Gel, 0.025%	Betamethasone benzoate
Westcort Ointment, 0.2%	Hydrocortisone valerate
CLASS V – Lower Mid-Strength	
Cordran Cream/Lotion/Tape, 0.05%	Flurandrenolide
Cutivate Cream, 0.05%	Fluticasone propionate
DermAtop Cream, 0.1%	Prednicarbate
DesOwen Ointment, 0.05%	Desonide
Diprosone Lotion, 0.05%	Betamethasone dipropionate
Kenalog Lotion, 0.1%	Triamcinolone acetonide
Locoid Cream, 0.1%	Hydrocortisone butyrate
Pandel Cream, 0.1%	Hydrocortisone probutate
Synalar Cream, 0.025%	Fluocinolone acetonide
Uticort Cream/Lotion, 0.025%	Betamethasone benzoate
Valisone Cream/Ointment, 0.1%	Betamethasone valerate
Westcort Cream, 0.2%	Hydrocortisone valerate
CLASS VI – Mild	
Aclovate Cream/Ointment, 0.05%	Alclometasone dipropionate
DesOwen Cream, 0.05%	Desonide
Synalar Cream/Solution, 0.01%	Fluocinolone acetonide
Tridesilon Cream, 0.05%	Desonide
Valisone Lotion, 0.1%	Betamethasone valerate
CLASS VII – Least Potent	
Topicals with hydrocortisone, dexamethasone, methylprednisolone and prednisolone	

Table 2
Classification of TG by potency according to the British National Formulary (BNF)

POTENCY	% w/w	TG
Mild		Hydrocortisone
	1	Hydrocortisone acetate
	0.25	Methylprednisolone
	0.05	Alclometasone dipropionate
	0.01–0.1	Dexamethasone
	0.0025	Fluocinolone acetonide
	0.75	Fluocortyn butyl ester
	0.5	Prednisolone
Moderate	0.05	Clobetasone butyrate
	0.02	Triamcinolone acetonide
	0.005	Fluocinolone acetonide
Potent	0.05	Betamethasone diproprionate
	0.1	Betamethasone valerate
	0.025	Fluocinolone acetonide
	0.1	Hydrocortisone 17-butyrate
	0.05	Halometasone monohydrate
	0.1	Diflucortolone valerate
Very Potent	0.1	Halcinonide
	0.05	Clobetasol propionate

either the solubility of the drug in the SC, or its diffusivity across the SC.

TG are formulated in a variety of vehicles, including ointments, creams, lotions, gels and, more recently, foams. As mentioned above, the vehicle has a great influence on penetration into the SC and consequently on the bioavail-

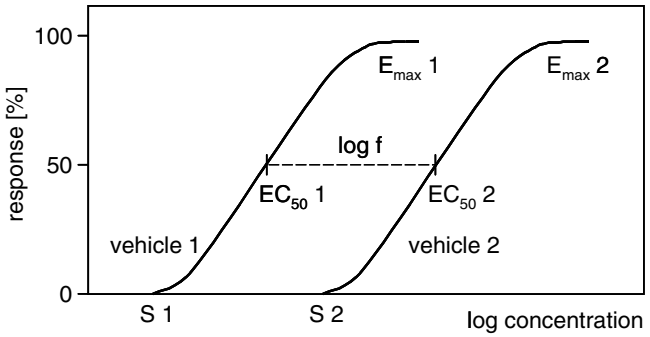

Fig. 7. Typical 'dose–response' curves of two vehicles.

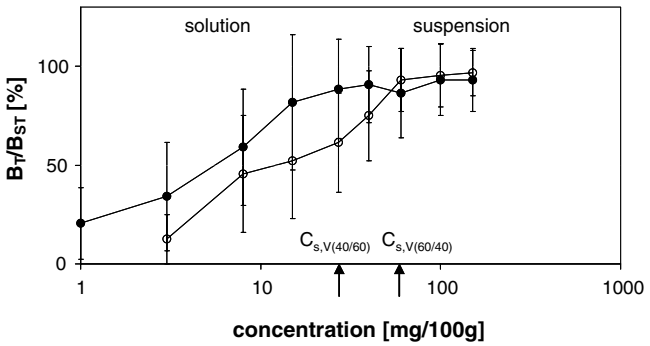

Fig. 8. Dose–response relationship of betamethasone 17-benzoate in two vehicles; neutral oil/paraffin 60/40 (○) and neutral oil/paraffin 40/60 (●). Mean ± SD, $n = 17$–18. Adapted from Lippold [79].

ability and potency of the glucocorticoid [80,81]. Ointment formulations are generally more potent than creams containing the same drug presumably due to their occlusive effect on the skin which may increase SC hydration and enhance drug transport [48,82,83]. Ointments are preferred for infiltrated, lichenified lesions, whereas creams are preferred for acute and subacute dermatoses. Lotions and gels are suitable for the treatment of scalp psoriasis. The novel, thermolabile, low-residue foam formulations, available for betamethasone 17-valerate and clobetasol propionate, are safe and effective in the treatment of psoriasis affecting scalp and non-scalp regions of the body. The foam formulations are associated with better patient compliance and improvements in quality of life [84,85].

The activity of a TG formulation can be enhanced by adding a chemical penetration enhancer which may result in an increase of drug delivery into and through the SC.

Various studies, using a vasoconstrictor assay, have shown that huge differences existed between generic and original formulations containing the same glucocorticoid in the same concentration in different vehicles [50,86]. By altering the vehicle, betamethasone dipropionate, at a concentration of 0.05%, has been formulated into four different potency groups (Table 1). Class I contains a cream (Diprolene® cream 0.05%) which is a modified vehicle high in propylene glycol. Class II includes an ointment (Diprosone® ointment 0.05%). Class III contains another cream (Diprosone® cream 0.05%) with less propylene glycol than

the Class I formulation. Class V incorporates betamethasone dipropionate in a lotion (Diprosone® 0.05% lotion).

Kinetic considerations of the penetration process differentiate between solution and suspension-type formulations [87,88]. With suspensions the rate of penetration should be independent of the vehicle (as long as the formulation does not change the solubility of the drug in the SC, or its diffusivity across the SC), the solubility of the drug therein, and the amount of incorporated drug as long as significant depletion does not occur over time [87]. However, in the case of solution-type formulations, the vehicle has an enormous influence on the rate of penetration. High drug solubility in the vehicle and a low partition coefficient between the SC and the vehicle lead to poor penetration of the drug into the SC and low bioavailability [77]. Therefore, it is important to know and control, where possible, the thermodynamic activity of the drug in the vehicle.

The dilution of commercially available TG formulations is a common practice which causes problems. The expectation of the prescribing physician is that dilution reduces the efficacy of the corticosteroid formulation and can be adjusted to the needs of the patient. However, the extent to which efficacy is reduced is not always proportional to the degree of dilution. For example, a fluocinolone acetonide cream (Synalar® cream), when diluted by up to 10-fold, resulted in no significant reduction in potency as assessed by the vasoconstrictor assay [89]. A betamethasone 17-valerate ointment (Betnovate® ointment) was diluted by a factor of 32 with no statistically significant difference in the blanching response [90]. These results strongly suggest that the drugs in the original formulations were present as suspensions and that, even upon substantial dilution, there remained some undissolved drug present. In other words the thermodynamic activity stayed at its maximum level; it follows that drug delivery into the skin was not changed and the pharmacological response was unaltered. Had the drug been present in the original vehicle as a solution, dilution would have lowered the thermodynamic activity and led to lower permeation and a lesser response. Care must also be taken when diluting formulations with a base which is not the same as the original vehicle. For example, Refai et al. showed that the *in vitro* permeation of hydrocortisone acetate is about 5 times lower after 1:2 dilution of Soventol® cream with a non-identical base (Fig. 9) [91]. Soventol® cream contains isopropyl alcohol and the penetration enhancer, isopropyl myristate. The reduced delivery is smaller than expected probably due to the reduced concentration of the enhancer in addition to the smaller thermodynamic activity of the drug.

Finally, it should be emphasized that the foregoing discussion is applicable specifically to intact, healthy skin, where penetration into and through the SC is the rate-limiting step. In the case of damaged skin, the release of drug from the formulation will determine uptake, and will be controlled by the characteristics of the vehicle.

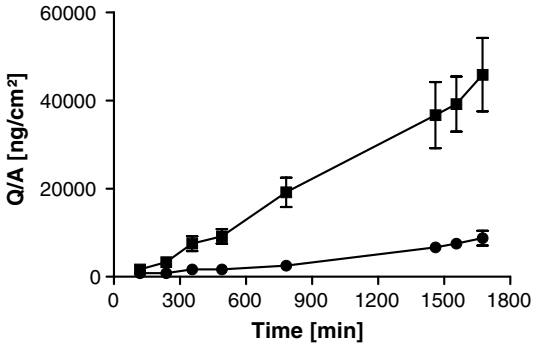

Fig. 9. *In vitro* SC permeation of hydrocortisone from 1% Soventol® cream (■), and Soventol® cream diluted with water-containing hydrophilic ointment 1:2 (●). Amount of drug permeating per unit area (Q/A) was plotted versus time. Adapted from Refai [91].

8. Bioavailability/bioequivalence testing

Bioavailability (BA) is defined as the "rate and extent to which the drug is absorbed from the formulation and becomes available at the site of action" (as stated in 21 CFR 320.1 [92]). Bioequivalence (BE) is defined as "the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study" (as defined in 21 CFR 320.24 [92]). As mentioned above, for TG the sites of action are the glucocorticoid receptors in the viable epidermis and dermis. Typical bioavailabilities are only a few percent of the applied dose. Several *in vivo* and *in vitro* methods have been employed to assess the BA/BE of TG, and are summarized in Fig. 10.

For the moment, the only acceptable methods to assess BA/BE of topically applied drug formulations are clinical trials between generic and original products and pharmacodynamic response studies. Comparative clinical trials are considered to be the 'gold standard', but these studies are relatively insensitive, costly, time-consuming and require large numbers of subjects [93]. In contrast, pharmacodynamic response studies are relatively easy to perform, expose the subjects to only a small amount of the formula-

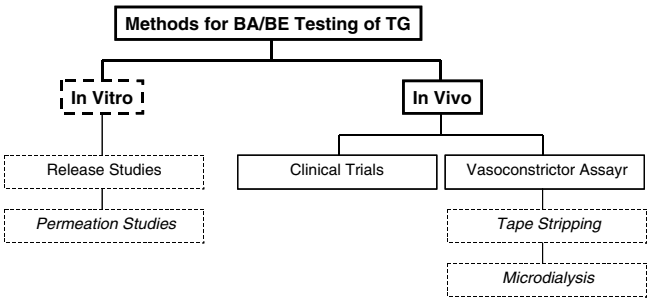

Fig. 10. Methods for BA/BE testing of TG, italicized legends signify those methods which are still under evaluation.

tion for a short period of time, are fairly reproducible, and require a relatively small number of subjects [94]. The TG pharmacodynamic response is the ability to produce vasoconstriction of the microvasculature of the skin, leading to skin blanching (whitening) at the site of application. This "vasoconstrictor assay" was first described by McKenzie and Stoughton in 1962 [95,96]. Since that time, the method has been modified and extended to provide a reliable means to test TG and their formulations. The intensity of skin blanching has been correlated with drug potency and the degree of drug delivery through the SC [97]. The blanching intensity has also been shown to correlate directly to clinical efficacy in patients with plaque psoriasis [48,82,98]. The vasoconstrictor assay has been used to measure the BA/BE of corticosteroid formulations in healthy volunteers [50,99,100] and has been adopted in 1995 for BE determination by the U.S. Food and Drug Administration (FDA), in a Guidance document "Topical Dermatologic Corticosteroids: *In vivo* Bioequivalence" [94]. This Guidance counsels both pilot and pivotal studies. The preliminary, pilot study is performed to establish the dose duration-pharmacological response relationship of a reference listed drug (RLD). The formulation is applied for various times (dose durations) up to 6 h to manipulate the amount of steroid delivered. At the end of the treatment period, the skin blanching response is measured with a chromameter over the next 24–28 h. From the resulting response versus time profiles, the areas above the response curves (AARC) are calculated and plotted as a function of dose duration to obtain dose/response-like relationships (in accordance with Fig. 6). From these profiles, the maximum AARC (= E_{max}) and the appropriate dose durations ED_{50}, D_1 and D_2 for use in the pivotal bioequivalence study are determined. ED_{50} is the dose duration required to achieve 50% of E_{max}, D_1 and D_2 correspond to one-half ED_{50} and two times ED_{50}, respectively. The pivotal bioequivalence study then compares the *in vivo* response of the test product with that of the RLD using appropriate statistical tools to document whether bioequivalence has, or has not, been achieved.

There remains a concern, however, that the design and analysis of the pilot study can influence the findings from the pivotal test [101,102], the role of the pilot investigation is therefore crucial. A critical factor, which is not specified in the Guidance, is the volume of formulation to be applied *in vivo*. It has been reasonably argued that the applied vehicle volume should be the same at all sites to ensure accurate and meaningful bioavailability data [102]. The situation becomes complex for preparations in which the drug is in solution and may therefore deplete over time, and their comparison with suspension-type formulations [102,103].

The chromameter has been adopted by regulatory agencies, such as the U.S. FDA, as the current standard for the measurement of corticosteroid-induced skin blanching. The chromameter quantifies the reflectance of a xenon source light pulse in terms of three measures: the L-scale (light–dark), the *a*-scale (red–green) and the *b*-scale (yellow–blue). These three values can be used to define a point in three-dimensional space that characterizes a color in absolute terms. The Guidance protocol suggests the use of only the *a*-scale values in quantifying the blanching response. The chromameter is viewed as an 'objective' measurement device compared to 'subjective' visual scoring. There are several reports comparing the chromameter with the visual technique [97,104–106], with other reflectance instruments [107], with laser Doppler velocimetry or, more recently, with digital image analysis [108,109]. Although, the chromameter has been adopted by the FDA as the current standard method for topical BE testing, it has been criticized for different reasons [101,104,110] and there are continuing efforts to examine alternative approaches. Nevertheless, despite its limitations, the vasoconstrictor assay remains the standard procedure to assess the BA/BE of TG.

Other pharmacodynamic effects that may be quantified are the vasodilatation (erythema) and skin temperature increase induced by nicotinic acid esters [111–115], and the response to local anaesthetic bases [116]. The reduction of methyl nicotinate-induced erythema by topical ibuprofen has also been correlated with the drug's concentration in the epidermis [117]. Topical retinoids have been shown to increase transepidermal water loss in a time and dose-dependent fashion [118,119]; however, the use of this effect for BE determination has not been confirmed or recommended [120].

In summary, therefore, apart from the vasoconstrictor assay, which is clearly restricted, at this time, to TG, there are currently no non-invasive or minimally invasive techniques for the assessment of BA/BE of topically applied drugs that are acceptable to the regulatory bodies. For all other topically applied drugs, comparative clinical trials are the only approved means with which to establish BE. In an effort to address this situation and to provide viable alternatives for BE determination, significant efforts are being directed to the dermatopharmacokinetic (DPK) approach, microdialysis and the use of *in vitro* experiments [121].

The DPK method uses tape-stripping to measure drug concentrations in the SC. The SC is collected by successive application and removal of adhesive tapes (Fig. 11) which are subsequently extracted and analyzed for the drug.

In theory, the DPK approach may be applied to all topical drugs. The principal assumption is that the amount of drug recovered from the SC, the usual barrier to percutane-

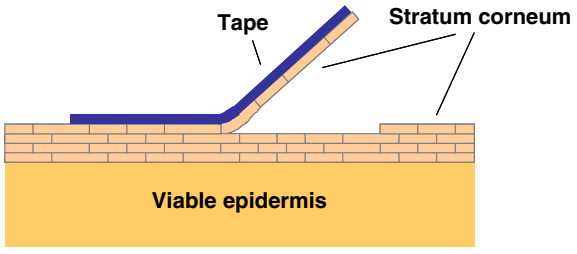

Fig. 11. Schematic representation of the tape-stripping technique.

ous absorption, is directly correlated with the amount reaching the target cells. A recently published comparison of DPK and skin blanching response following topical application of triamcinolone acetonide and betamethasone dipropionate has validated this hypothesis [48,122]. The DPK concept, which evolved from a series of earlier studies reported by Rougier et al. [123], was introduced in a Draft Guidance from the FDA in 1998 [124]. Over the following 4 years, a number of concerns were raised with respect to the relevance of the approach to diseased skin, to the applicability of the method to drugs which have rather specific sites of action (for example, the hair follicle), and to the reproducibility and practicability of the technique. In 2002, a comparative study using tretinoin gels was performed in two laboratories and produced conflicting results [125–127]. This led, in turn, to withdrawal of the Draft Guidance. Subsequently, the FDA has been critically re-evaluating DPK, with a view to improving sensitivity, to reducing complexity, and to validating the approach sequentially for specific drug classes. Other laboratories have also been contributing to this process and the value of improved DPK procedures has been demonstrated for the assessment of the BA of drugs whose site of action is the SC itself, such as: anti-fungal drugs [128–131], keratolytics [132–134], UVA/UVB filters [135–138], and antiseptics [139].

In principle, microdialysis allows continuous monitoring of the rate and extent of drug penetration into the skin. Via a probe (comprising a dialysis membrane) inserted into the dermis (Fig. 12), the *in vivo* sampling of endogenous and exogenous substances in the extracellular fluid is possible. The technique can directly measure, therefore, drug levels within the skin and comes closest, as a result, to offering information about BA in the target tissue. Further, the method may be used when the skin's barrier is disrupted and has been shown to be applicable to measurements on diseased skin [140,141]. Typically, the microdialysis probe (~200 μm diameter) is situated 1–3 mm beneath the skin surface and is perfused with a physiological solution (perfusate) at a volume flow rate of ~5 μl/min. The molecular weight cut-off of the dialysis membrane is in the order of 20 kDa. The exchange of substances across the dialysis membrane occurs by passive diffusion and depends on the relevant concentration gradient. These attractive features must be balanced against a number of significant challenges. First, and foremost, the technique is invasive and is difficult to perform; although very little biological fluid is removed, the mere insertion of the microdialysis probe can cause transient inflammation and the local release of various biological factors (e.g., cytokines) [142–144]. Second, the duration of a microdialysis experiment is necessarily limited for practical reasons, and this creates a problem for slowly or poorly permeating substances. Relatedly, recoveries are typically low: that is, the concentration of analytes in the perfusate is so low that analysis becomes difficult; this is particularly true for lipophilic drugs, such as betamethasone 17-valerate, or drugs which are highly protein-bound, which do not distribute significantly into the aqueous perfusate in the microdialysis probe [145,146]. Finally, to relate the amount of analyte in the perfusate to its real concentration in the skin, it is necessary to use a so-called "retrodialysis marker" (of similar physicochemical properties) to calibrate the recovery efficiency [147–149]. Overall, while microdialysis is an alternative option, the outstanding challenges preclude, at the present time, its use for routine BA/BE evaluation.

Finally, it is appropriate to describe briefly the value of *in vitro* procedures in the BA/BE evaluation of topical drug products. While the SUPAC-SS ("release test") procedure [150] is valuable as a tool for quality control, it is not appropriate for BE assessment except for very minor formulation changes. On the other hand, it may be said that *in vitro* skin permeation experiments have proven to be valuable guides in formulation development. However, the regulatory agencies (especially the FDA) have been reluctant to adopt such an approach for BA/BE determination. While there are reasonable concerns about the provision of human skin, of sufficient quality and quantity, for

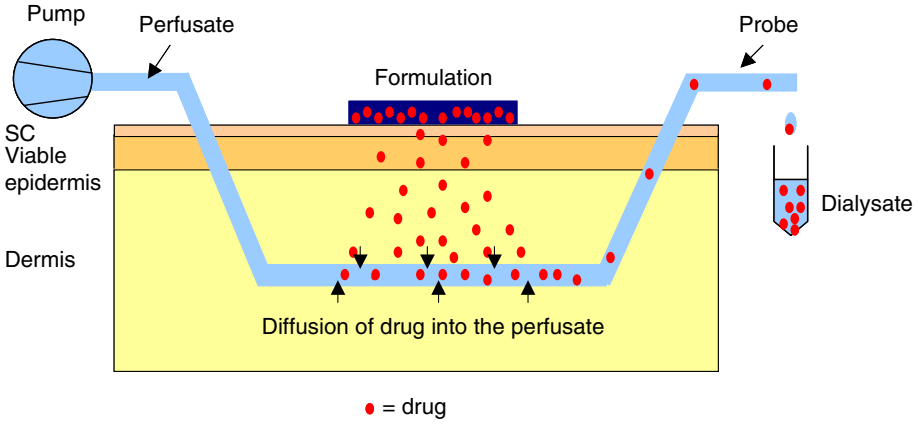

Fig. 12. Schematic representation of the principle of microdialysis. The probe is inserted in the dermis and is perfused at a flow rate controlled by the pump. Drug from the applied formulation permeates the SC and viable epidermis and eventually diffuses passively into the lumen of the membrane. The dialysate is sampled at fixed time intervals. Modified from Benfeldt [140].

routine use, there are probably situations, particularly for drugs of balanced lipophilicity/hydrophilicity, in which an *in vitro* skin penetration comparison of formulations would be perfectly adequate to judge BE.

9. Conclusions

Topical steroids are an integral part of dermatological therapy and will remain so in the foreseeable future. Despite their clinical success, however, the efficiency with which they are delivered remains poor and variable. Reasons underlying this sub-optimal situation are becoming clearer, and strategies to improve and normalize the performance of these powerful drugs are evolving. The development of new techniques, with which to quantify the rate and extent of steroid transport to the sites of drug action in the skin, is essential to the forward momentum of the field.

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