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Effects of Skin Occlusion on Percutaneous Absorption: An Overview

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Key Words

Barrier function · Enhancer · Occlusion · Percutaneous absorption-penetration · Skin hydration · Stratum corneum

Abstract

Skin occlusion produces profound changes, including hydration status, barrier permeability, epidermal lipids, DNA synthesis, microbial flora, and numerous molecular and cellular processes. It often, but not always, increases percutaneous absorption of applied chemicals. This overview focuses on the effect of skin occlusion on percutaneous absorption.

Introduction

Occlusion refers to covering skin by tape, gloves, impermeable dressings, or transdermal devices. Certain topical vehicles may also act as 'occlusive dressings' if they contain fats or some polymer oils, reducing water loss to the atmosphere. In healthy skin, the stratum corneum typically has a water content of 10–20% and provides a relatively efficient barrier against percutaneous absorption of exogenous substances [1]. Skin occlusion can increase stratum corneum hydration, and hence influence percutaneous absorption by altering partitioning between the surface chemical and the skin due to the increasing presence of water, swelling corneocytes and possibly altering the intercellular lipid phase organization, also by increasing the skin surface temperature, and increasing blood flow [2–4]. Occlusion may enhance drug efficacy [5–10]. Ac-

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tually, skin occlusion is a complex event producing profound changes and influencing skin biology as well as wound healing processes [11–27]. In general, occlusion can, with exceptions [2, 4, 28, 29], increase percutaneous absorption of topically applied compounds [30–42]; even a short-time (30 min) occlusion can result in significantly increased penetration and horny layer water content [43]. However, the effects of occlusion on absorption may also depend on the anatomic site as well as vehicle and penetrant [32, 37, 44].

Transdermal drug delivery systems have a high level of interest; in practice, skin is not readily breached at a therapeutic level because of barrier resistance. Various approaches have been employed to enhance absorption. Occlusion, perhaps due to its simplicity and convenience, has been extensively adopted to increase absorption. This overview focuses on the effect of occlusion on percutaneous absorption and summarizes related details.

Percutaneous Absorption in vitro

Gummer and Maibach [30] examined the penetration of methanol and ethanol through excised, full-thickness guinea pig skin in vitro at varying volumes and under a variety of occlusive conditions over a period of 19 h. Neither compound showed an increase in penetration with increasing dose volume. But occlusion significantly ($p < 0.01$) enhanced the penetration of both when compared to unoccluded skin. The nature of the occlusive material significantly influenced the penetrated amounts of both compounds, as well as the profiles of the amount penetrating per hour.

Hotchkiss et al. [31] evaluated absorption of model compounds, nicotinic acid, phenol and benzoic acid, and the herbicide triclopyr butoxyethyl ester with in vitro flow-through diffusion cells using rat and human skin. After application, the skin surface was unoccluded or covered with Teflon® caps as an occlusion device. The absorption of each compound across the skin and into the receptor fluid at 72 h was calculated. Occlusion significantly ($p < 0.05$) enhanced absorption of the model compounds, but varied with the compound and the skin (rat or human) used. They observed the effect of vehicle and occlusion on the in vitro percutaneous absorption of [methylene-^{14}C]-benzyl acetate (1.7–16.6 mg/cm^2) in diffusion cells using full-thickness skin from male Fischer 344 rats [32]. When benzyl acetate in ethanol was applied to the skin and occluded with Parafilm®, the extent of absorption at 48 h was not significantly different from unoccluded skin. But at 6 h, as the ethanol content of the application mixture was increased, the absorption of benzyl acetate through occluded skin was enhanced proportionally ($r = 0.99$). With phenylethanol as a vehicle, the extent of the benzyl acetate absorption through occluded skin at 48 h was significantly ($p < 0.05$) enhanced compared with unoccluded skin, but this did not correlate with the proportion of phenylethanol in the application mixture. With dimethyl sulfoxide as a vehicle, the extent of benzyl acetate absorption through occluded skin at 48 h was enhanced ($p < 0.05$) compared with unoccluded skin; when dimethyl sulfoxide content of the application mixture was increased, the absorption of benzyl acetate was enhanced proportionally. They concluded that occlusion often significantly enhanced absorption, but the effect varied with time and vehicle.

Roper et al. [33] tested the absorption of 2-phenoxyethanol applied in methanol through unoccluded rat and human skin in vitro in two diffusion cell systems over a period of 24 h. 2-Phenoxyethanol was lost by evaporation with both types of unoccluded

cells, but occlusion of the static cell reduced evaporation and increased total absorption to 98.8 ± 7.0%.

Treffel et al. [28] compared permeation profiles of two molecules with different physicochemical properties under occluded versus unoccluded conditions in vitro over a period of 24 h. Absorption was determined using human abdominal skin in diffusion cells under occluded and unoccluded conditions. Occlusion increased the permeation of citropten (a lipophilic compound; partition coefficient = 2.17) 1.6 times ($p < 0.05$) over unoccluded permeation. But the permeation of caffeine (an amphiphilic compound; partition coefficient = 0.02) did not show significant differences ($p = 0.18$) between occlusive and nonocclusive conditions. They confirmed the view that occlusion does not necessarily increase the percutaneous absorption of all chemicals [2, 4, 29].

Brooks and Riviere [44] utilized an isolated perfused porcine skin flap to determine the percutaneous absorption of ^{14}C-labeled phenol versus *p*-nitrophenol (PNP) at two concentrations (4 versus 40 μg/cm^2) in two vehicles (acetone versus ethanol) under occluded versus nonoccluded dosing conditions over 8 h. Occlusion increased the absorption, penetration into tissues, and total recoveries of phenol when compared to nonoccluded conditions. Absorption and penetration of phenol into tissues were greater with ethanol than with acetone under nonoccluded conditions, but the opposite was observed under occluded conditions. Phenol in acetone had a greater percentage of applied dose penetration into tissues at a low dose than a high dose, suggesting a fixed absorption rate. This was also seen for PNP, but only under occluded conditions. Neither phenol dose, vehicle, or occlusion had a significant effect on the labeled phenol seen in the stratum corneum or on time of peak flux, a finding which limits the usefulness of noninvasive stratum corneum sampling to assess topical penetration. Neither PNP dose, vehicle, nor occlusion had a significant effect on total recovery of labeled PNP. The authors suggested that comparative absorption of phenol and PNP are vehicle-, occlusion-, and penetrant-dependent.

Percutaneous Absorption in vivo

Animals

Bronaugh et al. [34] measured the percutaneous absorption of cosmetic fragrance materials, safrole and cinnamyl anthranilate, as well as of cinnamic alcohol and cinnamic acid, at occluded and nonoccluded application sites over a 24-hour period. They determined the absorption in the rhesus monkey in vivo, and also measured the absorption value through excised human skin in diffusion cell systems. Each radiolabeled compound was applied in an acetone vehicle at a concentration of 4 μg/cm^2. Occlusion was accomplished by taping plastic wrap to skin application site for in vivo experiments and by sealing the tops of the diffusion cells with Parafilm®. Occlusion of the application sites resulted in large increases in absorption, an effect consistent with the volatility of permeating molecules. When evaporation of the compounds was prevented, 75% of the applied cinnamic alcohol and 84% of the cinnamic acid were absorbed compared to 25 and 39%, respectively, without occlusion. In vitro experiments showed that the percutaneous absorption of these compounds was increased under occlusion in comparison to nonocclusion conditions (open to the air). The greatest difference between in vivo and in vitro absorption values occurred with safrole, which was the least well absorbed and the most volatile compound.

Subsequently, they determined the percutaneous absorption of the fragrance benzyl acetate (octanol-water partition coefficient = 1.96) and five other benzyl derivatives (benzyl alcohol, octanol-water partition coefficient = 0.87; benzyl benzoate, octanol-water partition coefficient = 3.97; benzamide, octanol-water partition coefficient = 0.64; benzoin, octanol-water partition coefficient = 1.35, and benzophenone, octanol-water partition coefficient = 3.18) in vivo in rhesus monkeys and human models [39]. Two occlusion methods (plastic wrap and glass chamber) were employed for 24 h. In general, absorption through occluded skin was high. Differences in absorption were observed between the methods. A low percentage absorbed for benzyl acetate was noted with plastic wrap compared to the unoccluded site, where glass chamber occlusion resulted in the greatest bioavailability. This discrepancy might be due to compound sequestration by the plastic. No correlations were found between skin penetration of these compounds and their octanol-water partition coefficients. Under unoccluded conditions skin penetration was reduced; there was great variability between compounds, possibly because of variations in the rates of evaporation from the application site.

Qiao et al. [35] described an in vivo model in female weanling pigs to quantify the disposition of parathion (PA) and its major metabolites for human dermal risk assessment following topical (occluded and nonoccluded dose of 300 μg, 40 μg/cm^2 on the abdomen and back) and intravenous (300 μg) ^{14}C PA. Total ^{14}C PA and its major metabolites in plasma, urine, blood, stratum corneum, dosed tissues, dosing device, and evaporative loss were determined. Occlusion enhanced the partition of both PA and PNP into the stratum corneum from the dosed skin surface, and also slowed down the distribution of PA and PNP in the local dosed tissues. Occlusion also altered the first pass biotransformation of PA in the epidermis. The authors further analyzed this data, focusing on a quantitation of the effects of application site (back versus abdomen) and dosing method (occluded versus nonoccluded) on in vivo disposition of both the parent PA and its sequential metabolites [36]. They concluded that occlusion not only increased ^{14}C absorption and shortened the mean residence time in most compartments but also altered the systemic versus cutaneous biotransformation pattern.

They investigated the effects of anatomical site and occlusion on the percutaneous absorption and residue pattern of total ^{14}C following topical application of PA onto four skin sites (300 μg/10 μCi; 40 μg/cm^2) in weanling swine using occluded and nonoccluded dosing systems [37]. Total excretion (% dose) in urinary and fecal samples after 168-hour dosing onto the abdomen, buttocks, back, and shoulder (n = 4/site), was 44, 49, 49, and 29% in the occluded system, and 7, 16, 25, and 17% in the nonoccluded system, respectively. The percutaneous absorption from the shoulder was much lower than that from the other three sites under occluded conditions. However, in the nonoccluded system, absorption from the abdomen was the lowest, with shoulder and buttocks being similar, and the back the highest. They suggested that anatomic site may influence the effects of occlusion.

Qiao et al. [38] utilized the same model to determine the pentachlorophenol dermal absorption and disposition from soil under occluded and nonoccluded conditions for 408 h. The absorption on occluded dosed sites (100.7%) was significantly enhanced (by more than 3 times, $p < 0.0005$) when compared to nonoccluded sites (29.1%).

Mukherji et al. [40] evaluated the topical application of 2′,3′-dideoxyinosine (ddI), a nucleoside analog used for treating patients with acquired immunodeficiency syndrome. A dose of ddI (approximately 180 mg/kg) dispersed in approximately 1 g ointment base was applied to the back of high follicular density and low follicular density rats with or without occlusion. At 24 h, the experiment was terminated and skin sections at the application sites were removed. After 24-hour topical application, average plateau plasma levels of about 0.6 μg/ml were achieved within 1–2 h and maintained for 24 h. Occlusion gave a more uniform plasma profile but did not increase bioavailability. The authors thought that the transfollicular absorption route for ddI did not have an important role due to the similar bioavailability in high follicular density and low follicular density rats.

Man

Feldmann and Maibach [41] correlated the increased pharmacological effect of hydrocortisone under occlusive conditions with the pharmacokinetics of absorption. ^{14}C hydrocortisone in acetone was applied to the ventral forearm. The application site was either unoccluded or occluded with plastic wrap. After 24-hour application, the unoccluded site was washed. At the occluded site, the wrap remained for 96 h post application before washing the site. The percent of the applied dose excreted into the urine, corrected for incomplete renal elimination, was 0.46 ± 0.2 (mean ± SD) and 5.9 ± 3.5 under unoccluded and occluded conditions, respectively. The occlusive condition significantly increased (10-fold) the cumulative absorption of hydrocortisone (total excretion: occluded = 4.48% versus nonoccluded = 0.46%). They noted that the difference of application duration (24-hour exposure on unoccluded site versus 96-hour exposure on occluded site) could influence the absorption as determined by the cumulative measurement of drug excreted into urine, but the significant difference in percent dose at 12 and 24 h between unoccluded and occluded was not expected to be dependent upon differences in washing times. Malathion, a pesticide, was intensively studied to determine the effect of duration of occlusion [45]. In as little as 1 h there was a significant increase in penetration (13% of absorption), and in 2 h 17%; in 4 h this was 24% and in 8 h 39%.

Ryatt et al. [42] developed a human pharmacodynamic model to measure the enhanced skin penetration of hexyl nicotinate (HN) using laser Doppler velocimetry (LDV). Before applying HN, the application site was either untreated (control) or subjected to one of four 30-min pretreatments: (a) occlusion with a polypropylene chamber; (b) occlusion (as in a) in the presence of 0.3 ml of the vehicle; (c) occlusion (as in a) in the presence of 0.3 ml of the vehicle containing 25% 2-pyrrolidone, and (d) occlusion (as in a) in the presence of 0.3 ml of the vehicle containing 25% laurocapram (1-dodecylhexahydro-2H-azepin-2-one). The onset of action, time to peak, peak height, area under the curve (AUC), time course, and magnitude of the LDV response were calculated. The onset of action and time to peak were significantly shortened, and the peak height and AUC significantly increased with pretreatments a–d (i.e., under occlusion conditions). Ryatt et al. [43] explored the relationship between increased stratum corneum hydration by occlusion and enhanced percutaneous absorption in vivo in man.

Percutaneous absorption of HN was monitored noninvasively by LDV following each of three randomly assigned pretreatments: untreated control, 30-min occlusion with a polypropylene chamber and 30-min occlusion followed by exposure to ambient conditions for

1 h. Stratum corneum water content after the same pretreatments was measured with the dielectric probe technique. The local vasodilatory effect of the nicotinic acid ester was quantified using LDV by the onset of increased blood flow, time of maximal increase in response, magnitude of the peak response and the area under the response time curve. A 30-min period of occlusion significantly shortened ($p < 0.05$) both the time of onset of the LDV-detected response to HN and the time to peak response when compared to the untreated controls. The stratum corneum water content values showed the same pattern, where the horny layer water content after 30-min occlusion was significantly elevated ($p < 0.001$). There was a significant correlation between stratum corneum water content and area under the LDV response-time curve after 30-min occlusion ($r = 0.8$; $p < 0.05$).

Bucks et al. [29] measured the percutaneous absorption of steroids (hydrocortisone, estradiol, testosterone, and progesterone) in vivo in man under occluded and 'protected' (i.e., covered, but nonocclusive) conditions. The ^{14}C-labeled chemicals were applied in acetone to the ventral forearm of volunteers. After vehicle evaporation, the site was covered with a semirigid polypropylene chamber for 24 h. The intact chambers were employed as the occlusion condition and by boring several small holes through the chamber as the 'protected' conditions (i.e., the roof of the chamber was covered with a piece of water-permeable membrane). Urine was collected for 7 days post application. Steroid absorption increased with increasing lipophilicity up to a point, but the penetration of progesterone (the most hydrophobic analog studied) did not continue the trend and was presumably at least partly rate-limited by slow interfacial transport at the stratum corneum. Twenty-four-hour occlusion significantly increased ($p < 0.01$) percutaneous absorption of estradiol, testosterone, and progesterone but did not affect the penetration of hydrocortisone. Absorption of the more lipophilic steroids was enhanced by occlusion but not that of the most water-soluble steroids (i.e., hydrocortisone). Table 1 summarizes the data of the effect of occlusion on percutaneous absorption.

Discussion

Skin, particularly the stratum corneum, serves as a barrier that prevents or limits the entrance of substances from the environment, and also modulates the balance of water loss from body fluids. Occlusion has the immediate effect of completely blocking diffusional water loss [22]. The consequence is to increase stratum corneum hydration, thereby swelling the corneocytes, and promoting the uptake of water into intercellular lipid domains [2, 4]. Occlusion can increase stratum corneum water content from a normal range of 10–20% up to 50% and can increase the skin temperature from 32 to 37°C [2, 4]. Occlusion also prevents the accidental wiping or evaporation (volatile compound) of the applied compound, in essence maintaining a higher applied dose [46]. In addition, it has a reservoir effect of the drug in penetration rates as a result of hydration [47]. Initially, a drug enters the stratum corneum under occlusion. After the occlusive dressing is removed, and the stratum corneum dehydrates, the movement of the drug slows and the stratum corneum becomes a reservoir [46, 47]. Hydration increased the penetration of lipid-soluble, nonpolar molecules but had less effect on polar molecules [28, 29]. The absorption of more lipophilic steroids was enhanced by occlusion but not that of the most water-soluble steroids (i.e., hydrocortisone) [29]. It is implied that the rate-determining role of the

Table 1. Summary of the effect of occlusion on percutaneous absorption

Models			Compounds	Results	References
in vitro	in vivo				
	animals	humans			
Guinea pig skin			methanol, ethanol	enhanced the penetration of both chemicals	30
Human skin			citropten, caffeine	increased the permeation of citropten (lipophilic compound) but not that of caffeine (amphiphilic compound)	43
Rat and human skin			nicotinic acid, phenol, benzoic acid, and triclopyr butoxyethyl ester	significantly enhanced the percutaneous absorption of the compounds, but varying with the compound under study and the skin (rat or human) used	31
Rat skin			benzyl acetate in different vehicles (in ethanol, phenylethanol, and dimethyl sulfoxide)	significantly enhanced absorption, but the effect varied with time and vehicle	32
Isolated perfused porcine skin flap			phenol and PNP in two different vehicles (acetone and ethanol)	increased the penetration of both compounds, but penetration was also affected by different vehicles	44
Rat and human skin			2-phenoxyethanol applied in methanol	reduced evaporation and increased total absorption	33
Human skin	rhesus monkey		safrole, cinnamyl anthranilate, cinnamic alcohol, and cinnamic acid	resulted in greater permeation of all of the compounds	34
	weanling pigs		PA and its major metabolites	increased the absorption and shortened the mean residence time; the effect of occlusion on percutaneous absorption was affected by anatomical site difference	35–37
	weanling pigs		pentachlorophenol	the absorption on occluded dosed site was significantly enhanced (by more than 3 times) when compared to nonoccluded site	38
	rhesus monkeys	humans	benzyl acetate and five other benzyl derivatives	increased the penetration with variability between compounds	39
	rats		2′,3′-dideoxyinosine	gave a more uniform plasma profile but did not increase the bioavailability	40
		humans	hydrocortisone	significantly increased the cumulative absorption	41
		humans	hexyl nicotinate	significantly increased the peak height and AUC, while the onset of action and time to peak were significantly shortened; also showed a significant correlation between water content and area under the LDV response-time curve	42, 43
		man	hydrocortisone, estradiol, testosterone, and progesterone	significantly increased percutaneous absorption of estradiol, testosterone, and progesterone but did not affect the penetration of hydrocortisone	29

sequential steps involved in percutaneous absorption can be revealed by experiments of the type described using related series of homologous or analogous chemicals. However, a trend of occlusion-induced absorption enhancement with increasing penetrant lipophilicity as apparent [28, 29]. An earlier report by Feldmann and Maibach [41] observed an increase in percutaneous absorption of hydrocortisone under occlusion conditions. Bucks et al. [2] and Bucks and Maibach [4] have explained these contrary data and suggested that they may be due to an acetone solvent effect. Topical application of acetone can disrupt the barrier function by extracting stratum corneum lipids [48, 49]. It is conceivable that 1 ml of acetone over an area of 13 cm^2 might compromise stratum corneum barrier function and hence increase the penetration of hydrocortisone under occlusion conditions. Experimental data are required to clarify this issue.

In practice, increasing the skin penetration rates of applied drugs is far from simple. Skin barrier function can be ascribed to the macroscopic structure of the stratum corneum, which consists of alternating lipoidal and hydrophilic regions. For this reason, physicochemical characteristics of the drug, such as the partition coefficient, structure, and molecular weight, play an important role in determining the facility of percutaneous absorption [50, 51]. Another factor to consider in transdermal drug delivery is the vehicle in which the drugs is formulated, as it acts on drug release from the formulation [32, 44]. Moreover, vehicles may also interact with human stratum corneum, thereby affecting its barrier function. Surfactants and penetration enhancers are well-known examples. Subsequently, dosing conditions, such as humidity, temperature and occlusion, also have their impact on the actual input (rate) of drug through human skin.

In conclusion, occlusion increases the percutaneous absorption of many but not all compounds. The effect of occlusion on percutaneous absorption may be also affected by the physicochemical properties (such as volatility, partition coefficient, and aqueous solubility), anatomical site, and vehicle.

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