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Effect of occlusion on the percutaneous penetration of linoleic acid and glycerol

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Abstract

The effect of occlusion on the in vitro percutaneous absorption of linoleic acid was investigated. A greater skin concentration of linoleic acid from an ethanolic vehicle was observed in non-occluded experiments compared with occluded experiments ($P < 0.05$). Such changes were not observed as consistently when ethanol was replaced with a less volatile organic solvent (cyclomethicone). These observations were attributed to the increase in the concentration gradient due to the unimpeded evaporation of volatile solvents, which provided a greater driving force and enhanced non-occluded delivery in these systems, compared with occluded systems. Conversely, the percutaneous absorption of a polar material (glycerol) from an aqueous solution did not yield any such differences. While more conclusive comparisons between volatile and non-volatile solvents and penetrants would be required to substantiate fully these comparisons, it is apparent that non-occlusion of volatile solvents may enhance percutaneous absorption. The physicochemical properties of the penetrant, for example its natural state at skin temperature (i.e. solid or liquid) may further determine the degree of enhanced percutaneous absorption compared with occluded environments.

Keywords: Occlusion; Percutaneous absorption; Linoleic acid; Glycerol; Porcine skin

1. Introduction

Occlusion of skin will substantially change many properties of the skin, including hydration, permeability of the skin barrier to some but not all exogenous chemicals, epidermal lipid composition, DNA synthesis, microbial flora, and other molecular and cellular processes (Zhai and Maibach, 2001). Of particular interest is the effect of occlusion upon percutaneous absorption. Occlusion of the skin surface leads to the entrapment of water, which would normally be lost to the surrounding environment. This results in an increase in skin hydration, particularly within the *stratum corneum* barrier, and a consequent swelling of corneocytes, an uptake of water in the intercellular skin lipids and the elevation of skin surface temperature from 32 to 37 °C (Bucks et

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al., 1989; Treffel et al., 1992). Occlusion is usually accomplished by the placement of a water-impervious dressing or drug delivery device (e.g. a transdermal patch) on the skin. Further, by virtue of their high viscosities and lipid content, certain drug vehicles may induce occlusion without the need for further dressings (Shelmire, 1960).

Occlusion of certain topical formulations has resulted in a significant increase of therapeutic activity for a wide range of drugs, including hydrocortisone (Scholtz and Calif, 1961; Sulzberger and Witten, 1961; Feldman and Maibach, 1967), other topical steroids (Vickers, 1963; Edwardson et al., 1993), tetracaine (McCafferty et al., 1989; Woolfson et al., 1990) and citrophen (Treffel et al., 1992). However, while Treffel and co-workers demonstrated that occlusion increased the transdermal flux of citrophen, a lipophilic molecule, no such increase in rate of delivery was observed for the amphiphilic molecule caffeine. Further, it was determined that occlusion and subsequent hydration did not increase the rate of delivery of small polar molecules (water, methanol or ethanol; Behl et al., 1980).

The vast majority of studies investigating percutaneous penetration are conducted under occlusion. This is perhaps due to the large number of studies indicating a link between an increase in flux across the skin and occlusion, and experimental requirements, such as the need to avoid evaporation from the donor cell compartment and maintain donor cell thermodynamics. As such, proportionately few drug delivery studies have investigated the effects of non-occlusion in modifying the delivery characteristics of a penetrant across the skin. Further, a substantial number of topically applied products, particularly cosmetics, are applied without occlusion. While such products are not intrinsically volatile they will undergo some loss of volatile constituents after their topical application, and any subsequent partition of formulation constituents into and across the skin will be conducted in a non-occluded environment.

Several naturally occurring oils, including almond, olive, maize and sunflower oils, are major sources of linoleic acid. Medicinally, it is employed to replenish, the skin barrier in cases of essential fatty acid deficiency, via topical application or intravenous fat emulsions (Press et al., 1974; Hunt et al., 1978; Elias et al., 1980; Houtsmuller and Beek, 1981). It is also employed widely in a variety of roles in cosmetic formulations. Therefore, the aims of this study were to compare the effect, which occlusion exerts upon the percutaneous permeation of the model penetrant linoleic acid, and from solvents of two different volatilities which are commonly employed in a range of topical formulations (ethanol and cyclomethicone). These studies were also compared with the delivery of glycerol, a model hydrophilic molecule.

2. Materials and methods

2.1. Materials

Linoleic acid, Dulbeco's phosphate buffered saline (DPBS) and Parafilm® were obtained from Sigma (Poole, Dorset, UK). Soluene 350, Emulsifier Safe Scintillation Cocktail and Hionic Flo Scintillation Cocktail were obtained from Packard Biosciences (Berkshire, UK). Lutrol F68 (Poloxamer 188) was obtained from BASF (Nottingham, UK). J-Lar tape was supplied by Stokvis (Permacel, NJ, USA). Ethanol RR and Parafilm® were obtained from Fisher Scientific (Loughborough, UK). Volatile Silicone DC245 was obtained from Dow Corning (Reading, UK). ^{14}C-linoleic acid and ^{14}C-glycerol were obtained from American Radiolabelled Chemicals (UK Agent; Tocris Cookson, Bristol, UK).

2.2. Methods

2.2.1. Preparation of porcine skin

Porcine skin, from an animal sacrificed previously for food use, was excised from a male pig (aged 16 weeks) as described previously (Woolfson et al., 1992).

2.2.2. In vitro diffusion cell experiments

Prior to their use, the Franz-type diffusion cells (Model FDC-400; Permegear Inc., USA) were rinsed twice and the receptor compartment filled with DPBS pre-warmed to remove any dissolved

oxygen. (For experiments involving linoleic acid only, the receptor cell was filled with 0.5% w/w Lutrol F68 in DPBS (Høelgaard and Møllgaard, 1982). The contents of the receptor cell were maintained at 37 °C and stirred continuously throughout the course of the experiment with a magnetic stirrer. The skin was cut to the appropriate size (approximately 3 × 3 cm) and transepidermal water loss (TEWL) was determined (Evaporimeter, Servo Med, Sweden) as an indicator of skin integrity (Idson, 1978). Assembly of the diffusion cell was then completed.

Three solutions were prepared for analysis. Linoleic acid (5% w/w) was incorporated into both ethanol and cyclomethicone (Volatile Silicone oil DC245). Glycerol (40% w/w) was dissolved in water. Samples were spiked with ^{14}C-glycerol or ^{14}C-linoleic acid of know specific activities, as appropriate.

A known volume (1 ml) of the radiolabelled formulations (ca. 1 μCi/ml) was applied to the skin within the donor cell (surface area 1.77 cm^2). For each experiment five replicates were carried out. In occluded experiments, the receptor cell sidearm and donor cell were both covered with Parafilm® whereas in the non-occluded experiments only the cell sidearm was sealed.

At the end of the 24-h experimental period, any excess formulation was pipetted from the skin surface. Any residue still remaining was removed by washing with water for 10 s in glycerol delivery experiments, and with ethanol:water (1:1) for 10 s in linoleic acid experiments. Excess liquid was pipetted from the skin surface and the skin surface was blotted dry with cotton wool. Five tape strips were taken from the skin surface. The remaining skin was cut into two horizontal sections (the top layer being 0.5 mm in thickness) via a dermatome (Nouvag AG/BA Ltd., Goldach, Switzerland). The tape strips, skin samples and the receptor cell contents were prepared for counting by dissolution in the appropriate scintillant and solvent.

2.2.3. Interpretation of results and statistical analysis

Results were recorded initially as disintegrations per minute by the scintillation counter (TriCarb 3100TR Liquid Scintillation Counter, Packard Biosciences, Berkshire, UK). These values were converted to concentrations, taking into account solvent and solute densities. Results from all experiments were analysed statistically by analysis of variance (ANOVA) methods (JMP v. 3.2 software). Concentrations of linoleic acid or glycerol in the donor cell and skin sections were compared by an ANOVA test followed by the Tukey–Kramer multiple comparison test. The receptor phase concentrations were compared by an ANOVA test.

3. Results

3.1. Linoleic acid

In general, similar trends were comparable between the four different experiments. A comparison of occluded versus non-occluded delivery of linoleic acid from ethanolic vehicles indicates that significantly more linoleic acid is delivered from the non-occluded system. Figs. 1 and 2 demonstrate that significantly more linoleic acid is found in the *stratum corneum* tape strippings (both combined and individually), the receptor phase and in both the upper and lower skin sections ($P < 0.05$ or < 0.01 in all cases) in the non-

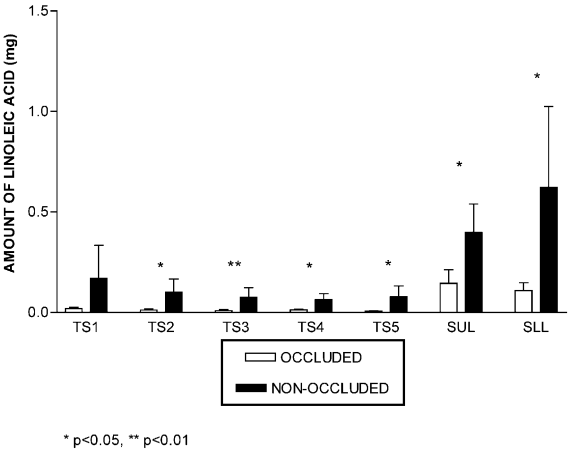

Fig. 1. Comparison of the amount of linoleic acid found on each of the tape strips and in the individual skin layers for both the occluded and non-occluded experiments from an ethanolic vehicle.

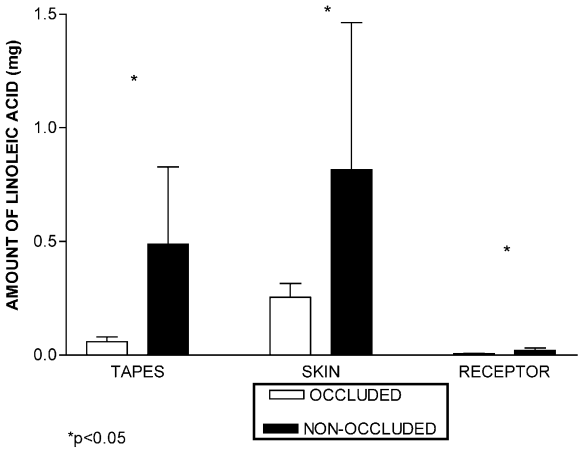

Fig. 2. Comparison of the amount of linoleic acid found on the tape strips, in the skin and in the receptor cell for both the occluded and non-occluded experiments from an ethanolic vehicle.

occluded experiments when compared with the occluded experiments.

Similar trends are observed for the non-occluded delivery of linoleic acid from a cyclomethicone solvent (Figs. 3 and 4). Significant differences are observed between occluded and non-occluded delivery into the delivery cell receptor phase ($P < 0.05$) and the amount delivered into skin (the total combined concentration in both sections of skin) ($P < 0.01$ for skin upper layers, and $P < 0.05$ for skin lower layers). While there are also some

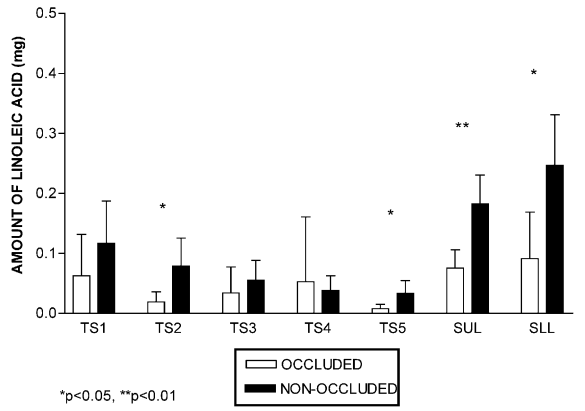

Fig. 3. Comparison of the amount of linoleic acid found on each of the tape strips and in the individual skin layers for both the occluded and non-occluded experiments from a cyclomethicone vehicle.

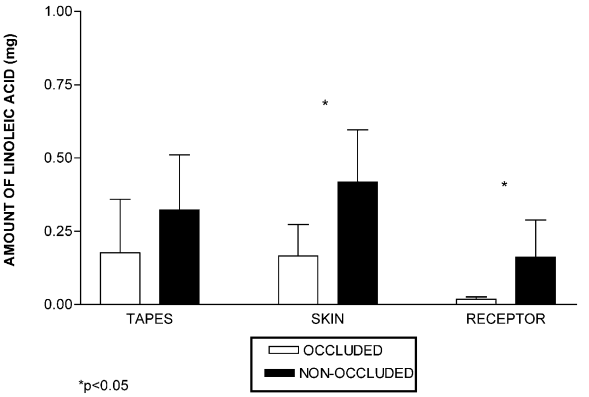

Fig. 4. Comparison of the amount of linoleic acid found on the tape strips, in the skin and in the receptor cell for both the occluded and non-occluded experiments from a cyclomethicone vehicle.

significant differences between the amounts of linoleic acid found in different tape strips ($P < 0.05$) the differences are not as large or consistently different as observed in ethanolic systems.

Comparisons between both occluded systems indicates that there are few consistent differences between the delivery from either solvent into any of the skin samples or receptor fluids analysed. Specifically, comparison of the ethanol occluded and cyclomethicone occluded experiments (Figs. 2 and 4) indicate that there are no significant differences in delivery of linoleic acid into the skin. However, significantly more linoleic acid passes into the receptor cell from the cyclomethicone vehicle than from the ethanol vehicle ($P < 0.05$). Comparison of the ethanol and cyclomethicone non-occluded experiments (Figs. 1–4) indicates that significantly more linoleic acid is delivered from the ethanol vehicle into the skin (skin upper layer, $P < 0.05$; total in skin $P < 0.01$), but that significantly more linoleic acid is delivered across the skin into the diffusion cell receptor compartment from the cyclomethicone vehicle ($P < 0.05$).

3.2. Glycerol

The tape strippings (Fig. 5) exhibit the same trend of depth-dependant concentration, with the majority of the analyte being detected in the upper

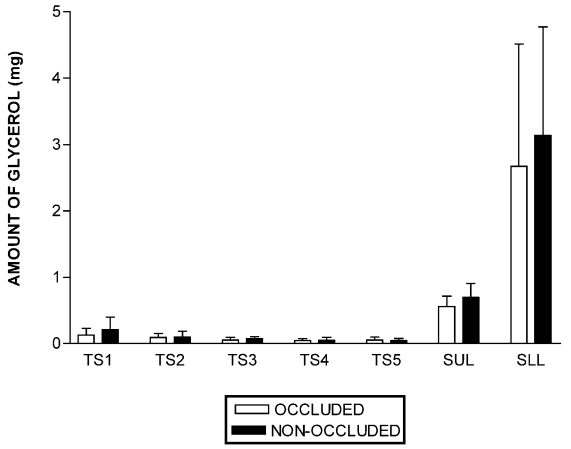

Fig. 5. Comparison of the amount of glycerol found on each of the tape strips and in the individual skin layers for both the occluded and non-occluded experiments.

tape strip and decreasing with depth, as observed with linoleic acid absorption experiments. There is no significant difference in the sum of all the tape strips, or comparisons of individual tape strips, between both experiments ($P > 0.05$ in all cases) (Figs. 5 and 6). No significant differences were found between the concentrations in the skin upper and lower layers for occluded and non-occluded experiments, or between the total concentrations of glycerol in these skin layers or in the receptor compartment of the diffusion cell (Fig. 6).

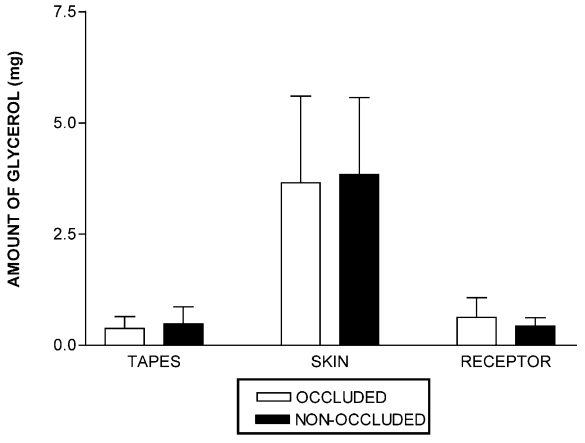

Fig. 6. Comparison of the amount of glycerol found on the tape strips, in the skin and in the receptor cell for both the occluded and non-occluded experiments.

4. Discussion

It is generally accepted that the occlusion of a topically applied formulation increases the penetration into and across the skin for most penetrants. However, occlusion does not always increase percutaneous absorption. Treffel, for example, demonstrated that while the rate of penetration of lipophilic citrophen increased under occlusion, amphiphilic caffeine exhibited no such increase when occluded. Therefore, the physicochemical properties of the penetrant and its vehicle may influence whether an exogenous chemical will penetrate at a different rate if occluded (Treffel et al., 1992).

Recently, Stinchomb and co-workers demonstrated that non-occlusion of formulations containing volatile solvents could have a significant effect on the delivery to the *stratum corneum* of a model penetrant, 4-cyanophenol (Stinchomb et al., 1999). The authors found that increasing the duration of exposure to aqueous solutions of 4-cyanophenol resulted in an increase in its uptake in the *stratum corneum*. The kinetics of uptake correlated well with predictive diffusive equations (i.e. Cleek and Bunge, 1993; Hansch et al., 1995; Cronin et al., 1999). Increasing the dose of this chemical in acetone also resulted in an increased uptake into the *stratum corneum*. However, this uptake eventually plateaued at a maximum level. Further, the amount of 4-cyanophenol taken into the *stratum corneum* was two to eight times greater than that from water following similar exposure times. The authors found that these results were consistent with findings in other studies which examined the uptake of hydrocortisone, mannitol, progesterone, ibuprofen and flurbiprofen (Akhter and Barry, 1985; Barry and Bennett, 1987). Similar findings have also been reported by Kondo and co-workers (Kondo et al., 1987). They concluded that although absorption from the aqueous vehicle eventually supersedes that from the acetone-deposited film, the initial unsteady state uptake was greatest from the evaporating vehicle solvent. The authors concluded that these observations were consistent with the increasing concentration gradient present on the skin surface when the volatile solvent was evaporating. This may provide a

greater driving force for the deposition of chemicals from such solvent systems, including the ethanolic systems applied herein. Thereafter, the solid drug was deposited on the skin surface, predominately bereft of solvent and unable to penetrate the skin to any further substantial degree. That concentrations of 4-cyanophenol in the *stratum corneum* rise with increasing dose only to a certain point and then plateau was attributed to the saturation of the drug in the *stratum corneum*, thus limiting any further increase in the concentration of 4-cyanophenol deposited in the skin. This is attributable to the solvent evaporation, an effect which would not be directly comparable with our studies but which may only be compared qualitatively, based on a comparison of the physicochemical properties of the various solvents.

The effect of non-occlusion with volatile solvents (ethanol and cyclomethicone) are consistent with those observed by Stinchomb and co-workers, in that the volatility of the solvents increases the concentration of the penetrant in the donor phase and enhances the deposition and delivery of linoleic acid into the skin (Stinchomb et al., 1999). However, one significant difference between both experiments is the nature of the penetrants. The materials examined by Stinchomb and co-workers, and other researchers (Barry and Bennett, 1987; Stinchomb et al., 1999) are solids in their natural state at the temperature of the experiments, whereas both linoleic acid and glycerol are both liquids under such conditions. Therefore, in the absence of any kinetic time-course studies it may be postulated that, whereas skin concentration rises rapidly to a plateau and ceases when the solid material is left on the skin surface predominately free of solvent. The absorption of linoleic acid and glycerol into the skin may also follow this initial trend but may well continue to penetrate the skin at a high rate as both liquids will still be able to penetrate the skin surface, unlike, for example, 4-cyanophenol. Such comments may not, however, consider fully the effects of skin secretions (e.g. sebum) present in vivo, which may provide a more complex environment in which to observe such phenomena compared with the in vitro environment employed in this study.

There is a readily observable trend regarding the effect of occlusion upon the amount of linoleic acid deposited in the skin (Figs. 1–4). However, it is widely established that ethanol will alter the composition of the skin barrier (Barry, 1983; Turraha and Ali-Fossi, 1987), and as such may enhance the penetration of topically applied exogenous chemicals. It is, therefore, not unreasonable to anticipate that a constant volume (1 ml) of ethanol, occluded on the skin surface for 24 h, would exhibit substantially greater skin absorption that a similar formulation under non-occluded conditions. However, the results presented in this study do not substantiate such a hypothesis. Figs. 1–4 demonstrate the opposite, with significantly more linoleic acid being delivered into and across the skin from non-occluded formulations, compared with occluded systems.

It would appear that two effects are influencing the delivery of linoleic acid in this case. Occlusion, and the constant presence of ethanol is generally considered to exert a large enhancing effect on skin absorption. It would appear that, in this study, the increase in the concentration of linoleic acid due to the evaporation of ethanol is the over-riding factor governing the delivery of this material. This results in an increase in the saturated solubility of linoleic acid in the donor phase.

Therefore, the results obtained, herein, would suggest that, for linoleic acid, an increase in the saturated solubility due to solvent evaporation is a more significant factor for percutaneous absorption than the barrier damage caused by the continued presence of ethanol in an occluded donor phase, similar to that observed by Stinchomb et al. (1999). This conclusion may be extended to materials with comparable physicochemical properties to linoleic acid.

Comparisons between the two solvents used are not so clear. There appears to be no discernible difference between the amount of linoleic acid delivered into the skin from either ethanol or cyclomethicone carriers in occluded experiments. However, significantly more linoleic acid is delivered across the skin (i.e. into the diffusion cell receptor phase) from the cyclomethicone solvent in both occluded and non-occluded experiments. This may be due to the relative polarity of both

solvents and reflect their partitioning into particular regions. For example, Lutrol F68 is a surfactant added to the receptor cell, after the method described previously (Høelgaard and Møllgaard, 1982), in order to improve the aqueous solubility of any linoleic acid penetrating across the skin. The addition of such agents to cell receptor phases is not uncommon; ostensibly this is undertaken to increase the solubility of highly hydrophobic materials in the aqueous receptor cell buffer. It may in this case result in a greater partitioning of linoleic acid delivered in a very hydrophobic solvent (cyclomethicone) into the receptor compartment rather than into the epidermal and dermal layers of the skin.

Significantly more linoleic acid is delivered into the upper layers of the skin from the ethanol system than from the cyclomethicone vehicle, in non-occluded experiments only. The upper section of the skin in these experiments consisted of a 0.5 mm layer from which five tape strips had been removed previously (the outermost layers of the *stratum corneum*). Therefore, this 'upper' layer may contain elements of the *stratum corneum* barrier as well as the remainder of the epidermis, and possibly a small component of the dermis. These findings may be due to the effects, which each solvent exerts upon the *stratum corneum*. As ethanol is recognised as being an effective enhancer of skin penetration (Barry, 1983; Turraha and Ali-Fossi, 1987), its presence may cause greater disruption to the skin barrier than the cyclomethicone solvent, ensuring that more material from the ethanolic carrier is carried into the skin than from the cyclomethicone vehicle.

No significant differences are observed between the delivery of glycerol in occluded or non-occluded experiments. When compared specifically to the linoleic acid experiments, this lack of differentiation may be attributed to the non-volatile nature of the solvent (water) and the polarity of glycerol, and its relative solubility in its vehicle. With no increased driving force from a changing solvent concentration in the donor phase relative to the occluded experiment, the concentration in both experimental protocols remains similar throughout the experiments. Consequently, the amount of glycerol delivered from these formulations was found to be not significantly different. However, this may also be a consequence of the high levels of glycerol in formulations examined in this study (40% w/w). The concentration employed in this experiments reflects the level of glycerol required to exert a clinically beneficial effect on *stratum corneum* integrity when applied topically (Fluhr et al., 1999). However, due to the limited nature of these studies and the different experimental conditions (i.e. receptor phase compositions) the value of a direct comparison with the linoleic acid results cannot be made. Rather, these results are presented to provide a qualitative comparison of the effects of occlusion upon volatile and non-volatile solvents for these very different systems and penetrants. Further work is required on these systems in order to make a definitive comparison of the effect of solvent volatility and penetrant lipophilicity upon their penetration into skin.

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