International Journal of Pharmaceutics 384 (2010) 67–72

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Oxybutynin permeation in skin: The influence of drug and solvent activity

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ARTICLE INFO

Article history:
Received 20 August 2009
Accepted 22 September 2009
Available online 30 September 2009

Keywords:
Octyl salicylate
Propylene glycol
Oxybutynin
Skin
Permeation
Supersaturation

ABSTRACT

The influence of degree of saturation (DS) of oxybutynin on permeation from octyl salicylate (OSAL) or propylene glycol (PG) vehicles was investigated, *in vitro*, in human skin. The permeation of OSAL and PG was also evaluated and the quantity of drug and solvent in the skin at the end of the diffusion study was measured. For OSAL the permeation of oxybutynin increased linearly with DS of drug for both 25 and 50% OSAL formulations. However, no differences were seen in oxybutynin permeation for formulations with the same DS but with different OSAL amounts, although the drug permeation was always slightly higher for 50% OSAL formulations. There was a decrease in the amount of OSAL extracted from skin with drug concentration (up to 5 DS). There was also a good correlation between the DS calculated from the amount of oxybutynin and OSAL extracted from the skin, and the actual DS of the formulation. In contrast oxbutynin DS did not affect PG permeation and there were no significant differences in oxybutynin permeation for the formulations with different DS. The lack of permeation enhancement for PG formulations appears to be related to PG depletion from the skin. The findings emphasise the importance of maintaining the drug in solution in order to achieve effective permeation from dermal and transdermal formulations.

1. Introduction

The use of supersaturated formulations to increase drug thermodynamic activity, as a strategy in transdermal drug delivery, was first considered by Higuchi (1960). Supersaturation is a state where the drug is at a higher concentration than its solubility limit and as a consequence of its higher thermodynamic activity, the flux should increase proportionally. The manipulation of drug thermodynamic activity, via supersaturation, to enhance drug permeation has been reported by a number of workers but few reports have investigated the influence of solvent/vehicle thermodynamic activity on drug permeation. This is surprising as the solvent/vehicle has been shown to dictate the extent and residence time of drug permeation *in vitro* (Francoeur et al., 1990; Trottet et al., 2004).

We have recently investigated the effect of the degree of drug saturation (DS) for the model drug, oxybutynin, on solvent and drug permeation in silicone membrane (Santos et al., 2009). Supersaturated residual phases of oxybutynin in octyl salicylate (OSAL) or propylene glycol (PG) were prepared by the method of solvent evaporation (Coldman et al., 1969). A decrease in OSAL permeation with the 5 DS formulation was observed in comparison with the 1 DS and 2 DS formulations, indicating a decrease in solvent activity with drug concentration. In addition, the drug transport from the 5 DS formulation was higher than from the 1 and 2 DS formulations but lower than predicted. Based on both solvent and drug permeation, this suggested that the low drug permeation observed with 5 DS resulted from a decrease in solvent thermodynamic activity rather than a decrease in solute activity as a result of drug crystallisation. For PG formulations, the PG permeation remained unaffected with increasing DS of the formulation, up to 5 DS.

In this paper we have extended the previous studies with OSAL and PG in silicone to human skin. The objectives were, firstly, to investigate the effect of drug concentration on OSAL thermodynamic activity, *in vitro*, in human skin and secondly, to compare the results for OSAL and PG with the data from the previous silicone studies. In order to achieve this, the permeation of OSAL in the absence and presence of drug was evaluated. Additionally, the quantity of drug and solvent (OSAL or PG) in the skin at the end of the diffusion study was evaluated using mass balance studies.

2. Materials and methods

2.1. Materials

Oxybutynin free base was a gift from Acrux, Ltd (Australia). OSAL and PG were purchased from Sigma (Australia). Phenylboronic acid and 1,2 butanediol, used for GC analysis as derivatisation and internal standard reagents, respectively, were produced by Fluka (Sigma,

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doi:10.1016/j.ijpharm.2009.09.043

Table 1
Oxybutynin concentrations (μmol/μl) in the 25% PG and OSAL formulations.

DS	PG, 25%	OSAL, 25%
1	0.13	0.20
2	0.26	0.39
5	0.65	0.98
7	–	1.37

United Kingdom). Polyethylene glycol 20 oleyl ether (PEG-20-OE) and orthophosphoric acid (85%, v/v) were purchased from Sigma (United Kingdom). All HPLC grade solvents were purchased from Fisher (United Kingdom).

2.2. Methods

2.2.1. Preparation of formulations

Supersaturated systems were prepared by the solvent evaporation method (Coldman et al., 1969). From the solubility values of oxybutynin in PG and OSAL (Santos et al., 2009) the amount required to saturate and supersaturate 100 μl of solvent was calculated. This amount was weighed (10 μg precision balance, Mettler AT261, Mettler Toledo, Inc., Switzerland) into a volumetric flask and then 100 μl of respective solvent was added to the volumetric flask and the volume made up, partially, with absolute ethanol. After dissolving the drug by sonication, the solution was left to equilibrate at room temperature for 30 min before completing the volume with ethanol. All solutions were freshly prepared on the day of the permeation studies. Table 1 lists all formulations used in the study.

2.2.2. Diffusion studies

Female abdominal tissue obtained from a single donor, with appropriate patient consent and institutional ethical approval, was stored at −20 °C until required. The permeation of oxybutynin, OSAL and PG across heat separated epidermis (Kligman and Christophers, 1963) was investigated using stainless steel flow-through diffusion cells (Hamilton Engineering, Australia) with an area available for drug diffusion of 1 cm^2. The receptor phase selected was 0.5% w/v PEG-20-OE in PBS pH 7.4 as no changes in the membrane permeability were observed compared with the buffer solution (PBS, pH 7.4) without surfactant (data not shown). The skin was mounted in the diffusion cells and equilibrated with the receptor solution 1 h prior to starting the study. To ensure sink conditions were maintained during the diffusion study, the receptor solution was pumped through the receptor compartment at a flow rate of 1 ml/h with a peristaltic pump (Watson Marlow, Stauff Corporation, Australia). At this flow rate, the receptor solution residing within the receptor compartment was replaced approximately 20 times/h (Traversa, 2005). A finite dose (3.6 μl/cm^2) of formulation was applied to the skin surface using a micropipette. At selected intervals, 0.2 ml samples were collected from the receptor compartment (Retriever II Fraction collector, Foss Pacific, Australia). Each experiment was conducted a minimum of four times. Prior to the start of the diffusion experiments, skin integrity was measured by impedance (Woo et al., 1992).

2.2.3. Mass balance studies

At the end of the permeation studies, the SC surface still mounted in the Franz cells was washed six times with 1 ml of an aqueous solution of 6% PEG-20-OE w/v. After washing (6× 1 ml), the membrane was blotted with filter paper. The membrane was then carefully stretched and the surface was swabbed with a cotton bud (dipped in the washing solution) in the parallel and perpendicular directions, in order to remove the excess liquid on the skin surface. The washing fractions were collected into the same vial, together with the cotton bud and the filter paper, and analysed by HPLC, after dilution with methanol. The membranes were then weighed, placed in an Eppendorf® vial and the drug was extracted twice with ethanol (2× 1 ml). Following application of 3.6 μl/cm^2 of a formulation of 50% OSAL v/v the OSAL recovery was 91 ± 4%. An aqueous 6% PEG-20-OE solution was chosen because of its solubilising capacity for drug (0.01 mmol/ml) and solvent (Walters et al., 1997).

2.2.4. HPLC and GC analysis

OSAL and oxybutynin were analysed by HPLC and PG was analysed by GC using the instrumentation and methodology previously reported (Santos et al., 2009).

2.2.5. Data analysis

Linear regression was used to assess the interval of skin permeation data (μg/cm^2) between 16 and 24 h and the slope estimated, giving a mean flux ($\bar{J}_{16-24\,h}$). Drug permeation from saturated residues was analysed using a finite dose model expressed as a Laplace transform as described previously (Santos et al., 2009). This allows the determination of P_1 and P_2, also known as the apparent partition and apparent diffusion parameters which are defined as follows:

$$P_1 = Kh \tag{1}$$

$$P_2 = \frac{D}{h^2} \tag{2}$$

The lag time t_{lag} and permeability coefficient k_p are further defined as

$$t_{lag} = \frac{1}{6P_2} \tag{3}$$

and

$$k_p = P_1 \times P_2 \tag{4}$$

Statistical significance was determined using one-way analysis of variance (ANOVA). Post hoc all pair wise multiple comparison of the means within different groups was performed using the Post hoc Bonferroni test. A probability of $p < 0.05$ was considered statistically significant. All results are presented as the mean ± SD, unless otherwise stated.

3. Results and discussion

3.1. Permeation of oxybutynin and OSAL from saturated and supersaturated residues

Permeation of oxybutynin and OSAL after the application of finite dose formulations with 25 or 50% OSAL v/v and with different DS were studied over 24 h. At the end of the experiment the skin surface was washed and the drug and solvent inside the skin was quantified, after extraction with ethanol.

3.1.1. Oxybutynin permeation: effect of drug and OSAL concentration

Fig. 1 shows the effect of DS and OSAL dose on the mean flux ($\bar{J}_{16-24\,h}$) of oxybutynin through human skin, following application of 3.6 μl/cm^2 of the formulations prepared with 25 and 50% OSAL at different DS. Table 2 gives the permeation enhancement ratio (ER_{per}) for each formulation which is the ratio between the mean flux of the formulation under study and the mean flux from the reference (saturated) formulation. A good correlation was found between the $\bar{J}_{16-24\,h}$ and the DS ($r^2 > 0.994$) for both formulations, but the ER_{per} obtained was slightly lower than predicted for the 25% OSAL formulation.