Pharmacokinetics of the Transdermal Reservoir Membrane System Delivering β-Estradiol: In Vitro/In Vivo-Correlation

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Purpose. The aim of our study was to investigate the high fluctuations of Estradiol (E2) plasma levels transdermally delivered in postmenopausal women by a commercially available membrane controlled reservoir system (MCRS).

Methods. The transdermal E2 flux either out of a complete MCRS or across its membrane out of defined ethanol water mixtures was determined, as well as E2 plasma profiles in 6 postmenopausal women produced by a MCRS.

Results. The transdermal in vitro E2 flux rate out of a complete MCRS, claimed to deliver 25 µg/day, increased steadily, reaching a maximum value of 2.06 ± 0.58 µg/h at 30 to 40 hours and decreased to a rate of about 0.5 µg/h from 60 to 90 hours. No statistically significant differences between plasma profiles calculated from the in vitro investigation and derived from a clinical study could be identified. The E2 flux in defined ethanol/water mixtures across MCRS-membrane, adhesive and skin layer increased with increasing ethanol concentrations up to a maximum of 227 ± 34 ng/cm²/h at an ethanol concentration of 62.5% (V/V) and decreased with further increase in the volume fraction of ethanol.

Conclusions. In vitro as well as in vivo investigations showed high fluctuation of E2 plasma profiles in postmenopausal women produced by the MCRS. These fluctuations are caused by a non-constant input rate of E2 which may be due to changing ethanol concentrations in the reservoir of the MCRS.

KEY WORDS: transdermal; estradiol; reservoir system; in vitro permeation; area under the curve; postmenopausal women.

INTRODUCTION

Estrogen replacement therapy (ERT) is widely accepted to relieve postmenopausal symptoms such as hot flushes, atrophic vaginal changes and sleep disturbances (1,2,3). This therapy reduces the risk of cardiovascular diseases (2) and stroke (4). Several routes of application as well as delivery systems for the administration of estradiol are available. The oral route is by far the most widely accepted one, whereas transdermal delivery forms have been introduced more recently (1,2).

The oral application requires high doses due to the metabolism in the intestinal tract (5) and liver, which leads to an unphysiologic ratio of E2 to the metabolite estrone (1). High concentrations of estrogens in the portal circulation enhance the synthesis of hepatic proteins, resulting for example in increased plasma levels of renin substrates and several hormone binding globulins (5) thus enhancing the risk to develop thrombosis.

The transdermal application avoiding the liver first pass effect reduces the required dose and provides E2 plasma levels comparable to the mid follicular phase (2) and does not induce synthesis of unwanted hepatic proteins.

It is widely believed among researchers, that E2 plasma levels with small fluctuations should be preferred, because there is a linear relationship between E2 plasma levels and reported reduction of symptoms like hot flushes (6). Also reduced estrogen plasma levels are associated with psychological symptoms (7). On the other hand, treatment related side effects such as breast tenderness and weight gain are frequently related to higher E2 doses (8). Additionally it was also recommended to raise plasma levels at the minimum effective dose to reduce the risk of breast and endometrial cancer (9).

A balance has to be achieved between the optimal therapeuetic effect and acceptable side effects. With this background in mind the pharmacokinetic profile may have important consequences in terms of efficacy, acceptability and compliance (8,10).

Currently two different types of transdermal patches for E2 drug delivery are available: Patches release E2 either from rather recently developed matrix type delivery system or from a so called membrane controlled reservoir system (MCRS), which is commercially available for more than ten years. In several clinical studies, where matrix patches were kinetically compared to the reservoir patches, the fluctuation in E2 plasma level in postmenopausal women produced by MCRS over the whole 96 h application period is significantly higher than those seen in matrix patches (11,12,13,14). The MCRS claiming to deliver 50 µg E2/day produces E2 plasma concentrations which increase to a maximum value of 80 to 100 pg/ml plasma at 30 to 40 hours post application followed by a decrease to values below 30 pg/ml at the end of the wearing period (11,13).

There have been speculations about the cause of the high fluctuation over the whole application period when MCRS patches are used.

MCRS patches consist of a reservoir which is separated from the skin by a rate controlling membrane composed of ethylene-vinyl-acetate copolymer (EVA) and a layer of adhesive. The reservoir contains a gelled solution of E2 in 95% (v/v) ethanol. The patch is attached to the skin by an adhesive consisting of a mixture of polysbutene and light mineral oil (1,15). One possible explanation for the abrupt drop in E2 delivery after 50 to 60 hours may be due to the loss of the cosolvent ethanol from the vehicle, because it is known that ethanol as well as estradiol delivered from the MCRS is absorbed by the skin (15). Taking this into account, the effect of ethanol supplementation to the patch on day three of use in postmenopausal women was investigated: A prolonged extension in E2 plasma levels associated with an increase in the area under the curve by 22 percent was observed (16). However, the rise to the maximum E2 plasma concentration 30 to 40 hours after the application could not be explained by these findings. Systematic investigations relating release properties of

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MRCS to plasma profiles are still lacking. These investigations would help to illustrate the cause of $E_2$ plasma level fluctuations either related to the drug delivery system or influenced by the physiology of women.

To conduct a systematic kinetic analysis under in vitro conditions using MCRS, the patch has to be attached completely onto human skin without compromising its integrity. This requires large pieces of excised human skin in the in vitro Franz cell type set up.

The aim of our investigation is therefore twofold: Firstly to determine the transdermal in vitro flux out of a MCRS across human skin, secondly, to investigate the influence of reservoir ethanol concentrations on the $E_2$ flux through the rate controlling membrane with adjacent adhesive layer and human skin.

To substantiate our in vitro investigation, an in vivo clinical study was conducted in postmenopausal women where a MCRS patch was applied for 84 hours to compare our in vitro results to in vivo findings.

MATERIALS AND METHODS

In Vitro Investigations

Materials

Estradiol (Sigma Chemical Co., St. Louis, USA) and ethanol (Lenz Chemie, Westerburg, Germany) were used as received. The Estraderm® TTS 25 transdermal delivery system (Batch No. 314600) containing 2.0 mg of $E_2$ had a nominal release rate of 25 mg per day and was commercially obtained. Gradient grade acetonitrile (LiChrosolv®, Merck KGaA, Darmstadt, Germany) was used in the preparation of the HPLC mobile phase and for solid phase extraction methods. Phosphate buffered saline (PBS) pH 7.4 was prepared from distilled water. All other reagents were of analytical grade.

Preparation of Epidermal Membranes

Human skin from breast reduction surgery was obtained from a local hospital. Skin preparation to split thickness was carried out as reported elsewhere (13). To test for skins integrity permeation experiments were conducted using $E_2$ as a marker with commercially available Franz diffusion cell type (Crown Glass Company, Inc., Somerville, N.J.).

In Vitro Determination of Flux Rates from the Patch

To investigate the transdermal $E_2$ flux out of a MCRS across excised human skin, a new diffusion cell was designed. The exact dimensions can be taken from Fig. 1. The inner glass cylinder which served as the acceptor phase had a diameter of 39 mm resulting in a volume of 56 ml. Sampling could be performed by two openings in the acceptor chamber (open circles, Fig. 1). During an experimental run openings were sealed by two stop-cocks. Sampling was performed by turning the complete diffusion cell, so that the openings were above the acceptor phase. A second cylinder served as a heating jacket. The experiments were performed at 37°C for 96 hr. The patch (3.8 cm in diameter) was attached to an adequate piece of human epidermis. A porous membrane (Polycarbonate, 47 mm, pore size 0.4 µm, Schleicher &Schuell, Dassel, Germany) was mounted between the skin and the diffusion cell to ensure permanent contact between skin and patch. The permeability of the membrane was estimated by a method previously described (17) and found to be in the range of $8.5 \pm 10^{-2} \text{ cm}^2/\text{sec}$, which is much higher than the permeability of human skin. Furthermore it was shown that adsorption of $E_2$ by the membrane was negligible (data not shown). The receptor compartment was filled with 56 ml of PBS and stirred at a constant speed of 250 rpm. The receptor fluid was periodically changed to maintain sink conditions. Epidermal pieces were optically examined at the end of each experiment for holes or signs of degradation. At predetermined time intervals samples of 25 ml were withdrawn and replaced with the same volume of PBS.

The whole sample was extracted using solid phase extraction columns (LiChrolut® RP 18, 100 mg, Merck KGaA, Darmstadt, Germany) under vacuum. Absorbed $E_2$ was eluted by 4 ml of acetonitrile. After evaporation of the solvent the residue was dissolved in 1 ml of acetonitrile and analyzed by HPLC. The recovery for the solid phase extraction method was found to be $92 \pm 6\%$. Amounts of $E_2$ released were calculated in each time interval after upwardly adjusting for dilution.

Calculation of $E_2$ Plasma Profiles

To compare the results from the in vitro investigation using the whole patch delivering 25 µg/day with data from the clinical study, the determined input rates were transformed to theoretical plasma concentrations using Eq. 1 (1):

$$\text{Flux} (\mu g \times d^{-1} \times \text{cm}^{-2} \text{ patch}) \times \frac{\text{patch surface area (cm}^2\text{)} \times [1 - P_{21}]}{C_{pl}(1 \times d^{-1})}$$

Where $C_{pl}(ss)$ is the steady state plasma concentration, $C_{pl}$ is the total plasma clearance and $P_{21}$ represents the ratio of the rate of conversion of $E_2$ to estrone to the input rate of $E_2$. When steady state is attained, then $P_{21}$ is constant and is referred to as the metabolic transfer coefficient (1). The total $E_2$ plasma clearance ranges from 615 to 790 l/d/m² (18,19), while the average body surface area of females is 1.6 m² (1). Therefore, an average plasma clearance of $1120 \text{ l/d}$ was assumed. The metabolic transfer coefficient $P_{21}$ has been reported to be 0.2 (20). Daily input rates for the investigated system delivering