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 (E_2) plasma levels transdermally delivered in postmenopausal women by a commercially available membrane controlled reservoir system (MCRS).

Methods. The transdermal E_2 flux either out of a complete MCRS or across its membrane out of defined ethanol water mixtures was determined, as well as E_2 plasma profiles in 6 postmenopausal women produced by a MCRS.

Results. The transdermal *in vitro* E_2 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 E_2 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 E_2 plasma profiles in postmenopausal women produced by the MCRS. These fluctuations are caused by a non-constant input rate of E_2 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 E_2 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 E_2 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 E_2 plasma levels with small fluctuations should be preferred, because there is a linear relationship between E_2 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 E_2 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 therapeutic 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 E_2 drug delivery are available: Patches release E_2 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 E_2 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 claimed to deliver 50 μg E_2/day produces E_2 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 E_2 in 95% (v/v) ethanol. The patch is attached to the skin by an adhesive consisting of a mixture of polyisobutene and light mineral oil (1,15). One possible explanation for the abrupt drop in E_2 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 E_2 plasma levels associated with an increase in the area under the curve by 22 percent was observed (16). However, the rise to the maximum E_2 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.

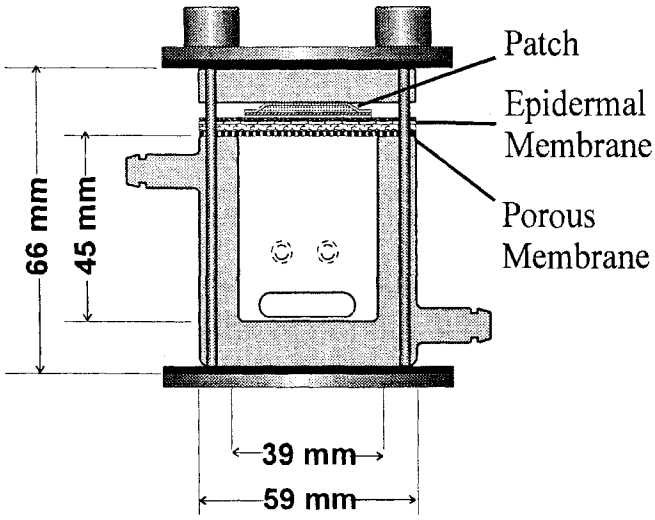

Fig. 1. Schematic view of the newly developed diffusion cell.

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 μg 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 ± 10^{-5} cm/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 ± 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):

$$Cp(ss) = \frac{\text{Flux}(\mu g \times d^{-1} \times cm^{-2}\ \text{patch}) \times \text{patch surface area } (cm^2) \times [1 - p_{21}]}{Clp(l \times d^{-1})} \quad (1)$$

Where Cp(ss) is the steady state plasma concentration, Clp 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/m^2 (18,19), while the average body surface area of females is 1.6 m^2 (1). Therefore, an average plasma clearance of 1120 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

25 μg/day were doubled to obtain the rates for a system with a nominal release rate of 50 μg/day, since it was shown that there is a linear relationship between patch surface area and obtained plasma concentrations (2). Finally the average E_2 baseline concentration of postmenopausal women of 10 pg/ml (2,21) was added.

Influence of Ethanol on Transdermal E_2 Flux Rates

MCRS membrane with adhesive layer was separated from the patch with the help of a pair of scissors. This part of the patch was attached to a piece of human skin. To investigate the E_2 flux through membrane, adhesive and skin layer in dependence on donor ethanol concentrations, this composite was mounted between two well stirred, 37°C water jacketed diffusion cell halves. The donor compartment, facing the membrane, consisted of 4 ml of saturated solutions of E_2 in defined ethanol/PBS co-solvent systems, while the receptor contained 4 ml of PBS. The receptor medium was periodically changed to maintain sink conditions. Samples of 1 ml were withdrawn from the receiver and replaced with receptor solution. The samples were assayed for E_2 by HPLC. Triplicate experiments were conducted for each study.

HPLC Assay

A high performance liquid chromatographic system (Merck-Hitachi L 6200 A, Merck KGaA, Darmstadt, Germany) equipped with an F-1050 fluorescence spectrophotometer and an AS 2000 A autosampler was used in this investigation. A LiChrospher® 100 RP 18 (5 μm, 250×4.6 mm i.d., Merck KGaA) was used as analytical column maintained at 30°C. The mobile phase consisted of acetonitrile/water (60:40) at a flow rate of 1.0 ml/min. The detector was set to an excitation wavelength of 225 nm with no emission filter used.

Clinical Study

Patients

Six healthy postmenopausal women participated in the study. Their mean age was 56 ± 5 years, with a median time since menopause of 9 ± 5 years. Postmenopausal status was confirmed in each case by a 17 β-estradiol (E_2) concentration below 20 pg/ml and a FSH level higher than 40 IU/l. This investigation followed the tenets of the Declaration of Helsinki (1964) and was approved by the Ethical Committee of the University Hospital in Frankfurt. Written informed consent was obtained from all women prior to their entry into the study.

Study Protocol

The study was performed using an open design in accordance with the Good Clinical Practice guidelines. A one week run-in period was followed by a 10.5 days treatment period and a final follow-up period of one week. During the treatment period the transdermal system was worn for three consecutive 3.5 day-periods. A MCRS containing 4 mg E_2 and releasing 50 μg/day (Estraderm® TTS 50, Batch No. 108400) was used in this study.

Design, study protocol and blood sampling was conducted as was reported elsewhere (13). Blood samples were assayed for E_2 by a capillary gas chromatography mass spectrometry method with negative ionisation mode (GC-MS), which is described elsewhere (13). The limit of quantification was 5 pg/ml for E_2.

Pharmacokinetic Parameters

The main pharmacokinetic parameters like c_{max} and t_{max} were calculated according to standard methods (22). The area under the E_2 plasma concentration curve ($AUC_{3\to78}$) was calculated using the linear trapezoidal rule for the time interval from 3 to 78 hours. The average concentration during the measurement interval, c_{av}, was calculated by dividing $AUC_{3\to78}$ by 75 h.

Statistical Comparison of E_2 Plasma Profiles In Vivo *and* In Vitro

$AUC_{3\to78}$, c_{max}, c_{av} and t_{max} were compared using Students t-test at 0.05 significance level (Statgraphics Plus, Statistical Graphics Corp.). For AUC values linear regression and correlation coefficient were calculated between non-normalised parameters.

RESULTS

To test excised human skins integrity, flux rates from saturated solutions of E_2 in water were determined. The permeability was found to be $6.7 \times 10^{-3} \pm 3.8 \times 10^{-3}$ cm/h (n=6), which is comparable to permeabilities reported by numerous investigators (23).

The cumulative amount of E_2 from a complete MCRS, claimed to deliver 25μg E_2 /day, across excised human skin in a newly designed Franz diffusion cell is depicted in Fig. 2. A sigmoidal curve can be identified, indicating that the release of E_2 into the receptor was lower at the beginning and the end of an experimental run and was largest 30 to 40 hours after the start. The receptor volume of 56 ml of the newly designed Franz diffusion cell was large enough to maintain sink conditions overcoming the problem of saturation of the acceptor phase (the solubility of E_2 in PBS is rather low: about 2.4 μg/ml). Transdermal E_2 flux rates in each respective time interval are

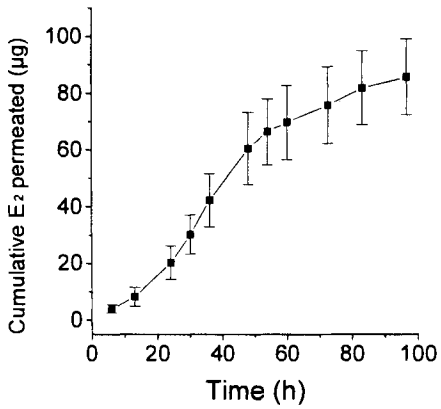

Fig. 2. Cumulative amount of E_2 diffused through the skin from the patch (nominal release rate: 25 μg/day). Each data point represents the mean and standard deviation of six determinations.

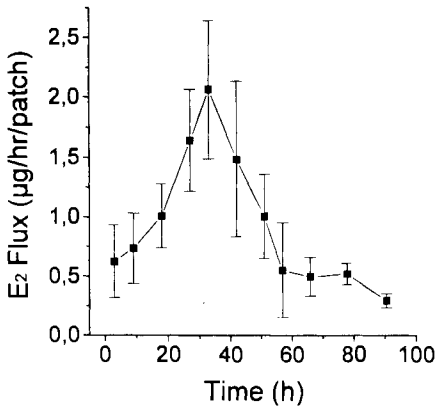

Fig. 3. *In vitro* flux of E_2 from the MCRS across human skin. Each data point represents the mean and standard deviation of six determinations.

shown in Fig. 3. These data were calculated from cumulative amounts penetrated under *in vitro* conditions (depicted in Fig. 2). The transdermal flux rate from the system 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, indicating high fluctuation. This non constant input rate of E_2 from the patch across human skin is in contrast to the constant delivery rate claimed by the manufacturer.

A clinical study was conducted with postmenopausal women receiving a MCRS with a nominal delivery rate of 50 µg/day. All patches were well tolerated and no side effects, systemically or locally, were reported.

After an increase the E_2 plasma profile curve reaches a relatively sharp maximum in the range of 80 pg/ml at about 30 hours post application followed by a decrease to plasma levels below 30 pg/ml after 60 hours. The E_2 plasma profiles experimentally obtained were compared with data from another clinical study (11). No statistically significant difference between the results of these investigations could be determined.

The E_2 plasma profiles calculated from *in vitro* data (taken from Fig. 3) are depicted in Fig. 4 and are compared to the E_2 plasma concentration profile obtained *in vivo*.

Similar profiles for both curves are observed, although the decrease for the data obtained *in vivo* is stronger than for the

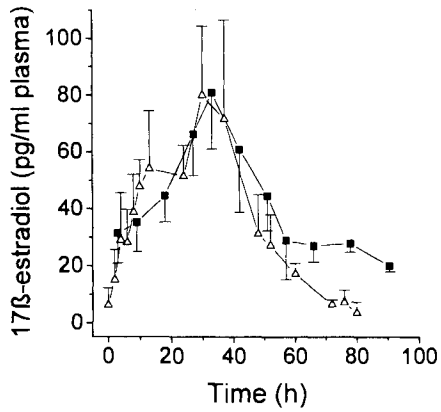

Fig. 4. Plasma E_2 concentrations measured before and during the third application of a MCRS in vivo (-Δ-) and calculated from *in vitro* determined input rates (-■-). Each data point represents the mean and standard deviation of six determinations.

calculated curve after 40 hours. This may be partially due to the quantification limit of the GC/MS method, because values below or near this limit were taken as zero. The main pharmacokinetic parameters were calculated for both profiles (Table 1).

An *in vitro/in vivo*-correlation based on AUC_{3-t} versus time comparison was conducted for the time interval from 3 to 78 hours post application. Each integral for each time interval of the calculated curve was compared to respective intervals of the *in vivo* curve. The comparison revealed a slope and a regression coefficient of 0.89 and 0.993, respectively, representing a good agreement between E_2 plasma profiles detected *in vivo* and predicted from *in vitro* data.

Since it is known that ethanol is released concomitantly with E_2 from the patch (15), we assumed that there should be a relationship between the ethanol concentration in the reservoir and the E_2 flux out of the system across human skin. To investigate this effect, the E_2 flux through a sandwich consisting of MCRS-membrane, adhesive and skin layer in dependence on ethanol concentrations in the donor was measured. The interdiffusion of ethanol and water across these barriers exists, but can be neglected at the present state of our investigations, because firstly the ethanol/PBS volume fraction in the donor phase can be judged as nearly constant within experimental time in the side-by-side *in vitro* diffusion cell, and secondly the diffused amount of ethanol into the acceptor phase is so low, that pure PBS solution can still be assumed. These permeation studies were conducted using saturated drug solutions to ensure that the permeant is at its maximum chemical potential. In the absence of any specific solvent/membrane interaction, the flux of E_2 from each co-solvent system ideally should be constant (24). However, the E_2 flux increased with increasing ethanol concentrations in the donor up to a maximum of 0.227 ± 0.034 µg/cm²/h at an ethanol concentration of 62.5 % (v/v) (Fig. 5). The permeation then decreased with further increase in the volume fraction of ethanol in the donor solution. From these findings it can be concluded that the transdermal E_2 flux out of a MCRS is strictly dependent on the ethanol concentration in the donor and therefore the penetration rate of ethanol from the MCRS.

DISCUSSION

Experiments *in vivo* as well as *in vitro* were conducted to investigate the kinetic performance characteristics of a commercially available membrane controlled reservoir system (MCRS). *In vivo* experiments were carried out in postmenopausal women applying a MCRS claimed to deliver 50 µg E_2/day. A maximum E_2 plasma concentration at about 30 to 40 h after patch application was identified, while the lower E_2 flux rate at the end of the wearing period leads to plasma levels below the therapeutical threshold of 30 to 40 pg/ml. This observation was confirmed by other clinical investigations in the past (11,13).

In a recently published clinical study two newly developed patches with matrix technology showed less fluctuating E_2 plasma profiles over the whole application period, however pronounced daily periodic changes in the E_2 plasma profiles (25). These daily variations could not be observed in the clinical study reported here using a MCRS.

We assumed that the fluctuations produced by the MRCS are so high, that the daily variations are difficult to detect.

Table 1. Pharmacokinetic Parameters of Plasma E_2 After Application of a MCRS *In Vivo* and Determined for the Calculated Plasma Profile

Parameter	Clinical study (n = 6) (mean ± Sd)	Calculated profile (n = 6) (mean ± Sd)	Statistical comparison (Student's t-test)
$AUC_{3\text{-}78}$(pg/ml*h)[b]	3033.42 ± 635.45	3411.81 ± 424.76	NS[a]
c_{max}(pg/ml)	88.94 ± 27.03	88.71 ± 13.78	NS[a]
c_{av}(pg/ml)	40.45 ± 8.47	45.49 ± 5.66	NS[a]
t_{max}(h)	30.7 ± 9.03	32.5 ± 5.5	NS[a]

[a] Not significant at 0.05 significance level.
[b] Abbreviations: $AUC_{3\text{-}78}$: area under the plasma concentration-time curve; c_{max}: mean maximum plasma concentration; c_{av}: average plasma concentration from 3 to 78 hours; t_{max}: time to c_{max}.

Recently an *in vitro/in vivo*-correlation for a matrix system delivering E_2 over a long period of time with a constant input rate based on experimentally determined E_2 fluxes across excised human skin showed no statistical significant differences between calculated and clinically determined E_2 plasma levels (13). Here, for the first time, an *in vitro/in vivo*-correlation for a MCRS with a non-constant input rate was conducted. The transdermal *in vitro* flux of E_2 out of a MCRS across excised human skin increased to a maximum value at about 35 hours after patch application and declined then until the end of the experiment. Calculated plasma profiles and E_2 plasma concentrations obtained in vivo were compared: No statistically significant difference between the two plasma profiles could be identified, showing that there is a possibility to directly predict plasma concentrations from *in vitro* investigations.

The *in vitro* method used here reflects recommendations for *in vitro* skin permeation tests of dermal and transdermal drug release products of the AAPS and FDA (26,27) concerning source and preparation of skin, the cell design and the experimental conditions. Two modifications though had to be made: Firstly, the surface area had to be increased to about 11cm^2 and secondly a supporting membrane had to be introduced between the skin membrane and the acceptor phase. The later assured contact between skin and patch during the experimental time. This additional membrane did not add a statistically significant diffusion barrier as well no adsorption of E_2 at the supporting membrane took place.

The decline in plasma levels at the end of one wearing period was interpreted by others to be due to the loss of the solvent and the enhancer ethanol from the patch (16). This does not explain, however, the increase to a maximum flux out of the system 30 to 40 hours after the application of the patch. Since both water and ethanol permeate the membrane/adhesive layer, changes must occur constantly in the alcohol to water ratio during the application of the patch. Fig. 5 shows the effect of changing the ethanol concentration in the donor on the transdermal flux of E_2: The release through membrane, adhesive and skin layer is maximum at ethanol levels of about 50 to 60 % (v/v) and is lowered if this ratio is altered in favor of water. This strongly suggests that changing ethanol concentrations in the reservoir of the patch might be responsible for the non constant transdermal E_2 input rate.

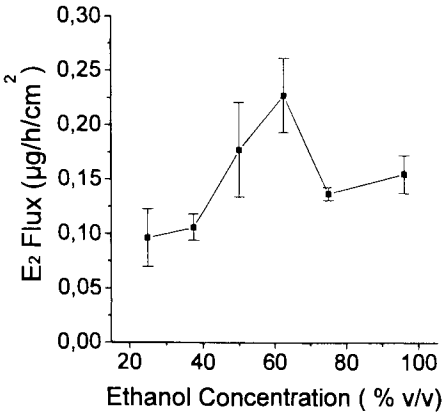

Fig. 5. E_2 flux through membrane, adhesive and skin layer as a function of ethanol concentrations in the donor compartment. Each data point represents the mean and standard deviation of three determinations.

The release profile of a MCRS conducted at the FDA by the paddle over disc method where no skin was employed (28) produced a similar sigmoidal cumulative amount versus time profile as in the experiments summarized in Fig. 2., but differs in two important points: Firstly the total amount released per cm^2 was more than 2.5 times higher in dissolution tests, and secondly, the ratio between results utilizing skin membrane and no skin membrane is constant from hour 48 to 96, however, changes from hour 3 to 48. This strongly indicates that the skin not only altered the absolute amount of E_2 released, but also had an influence on the release kinetics.

Variability associated with the source of skin and its preparation, and its cost, preclude the use of *in vitro* skin penetration testing for routine quality control (29), but it can be derived that the skin is a contributing factor in regulating the release of E_2 out of a MCRS and cannot be ignored if in vivo events are wished to be predicted.

Therefore, the method outlined here can be seen as a tool for the prediction of influences on the pharmacokinetic profile of a MCRS resulting from variations in the technology of the patch. *In vitro* and *in vivo* investigations suggest, that the constant delivery rate for the MCRS postulated by the manufacturer could not be confirmed.

It could thus be shown by these *in vitro* investigations that the MCRS produces fluctuating transdermal E_2 fluxes and biological factors in postmenopausal women are not responsible for this effect.

Further investigations are under way to specifically elucidate the contribution of the MCRS design to the fluctuations of E_2 transdermal flux rates.

CONCLUSIONS

In vitro as well as *in vivo* investigations were conducted with a commercially available membrane controlled reservoir

system (MCRS) to illustrate the high fluctuation of β-estradiol (E_2) plasma profiles in postmenopausal women. The transdermal E_2 flux rate out of a MCRS was investigated for the first time across excised human skin *in vitro*, increasing steadily, reaching a maximum value of 2.06 ± 0.58 μg/h at 30 to 40 hours and decreasing to a rate of about 0.5 μg/h from 60 to 90 hours. This fluctuating profile indicates that there is a non constant input rate of E_2 from the patch through the skin, which is in contrast to the constant delivery rate claimed by the manufacturer. Calculated plasma profiles from *in vitro* data showed a good correlation to data from a conducted clinical study, indicating that the *in vitro* method is suitable for the prediction of plasma levels from *in vitro* investigations. Changing ethanol concentrations in the reservoir have a strong influence on the flux of drug through membrane, adhesive, and skin layer. This effect may be responsible for the observed unstable E_2 input rates out of a MCRS.

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