Cutaneous and Ocular Toxicology, 2009; 28(4): 171–175

informa
healthcare

RESEARCH ARTICLE

In vitro human topical bioactive drug transdermal absorption: estradiol

Malcolm M. Q. Xing [1,2], Xiaoying Hui[3], Wen Zhong[4], Ning Pan[5], Frank Yaghmaie[6], and H. I. Maibach[3]

[1]Department of Mechanical and Manufacturing Engineering, University of Manitoba, Winnipeg, Canada, [2]VA Tissue Engineering Lab, Department of Orthopedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, [3]Department of Dermatology, University of California, San Fransisco, CA, USA, [4]Department of Medical Microbiology and Department of Textile Sciences, University of Manitoba, Winnipeg, Canada, [5]Department of Biological Systems Engineering, University of California, Davis, CA, USA, and [6]Northern Californian Nanofabrication Center (NC2), Davis, CA, USA

Abstract

Use of the percutaneous route may avoid some of the undesirable side effects that occur following oral administration in estrogen replacement therapy. At present, knowledge of estradiol transdermal properties relating to delivery of drugs in the skin is lacking. One reason is that in the existing transport models of estradiol, the skin is regarded as a single layer. This study revealed a significant difference of effects on estradiol delivery in the 3 sublayers of the skin and has caused us to believe that if we can obtain information about the transfer properties of estradiol in human skin (3 sublayers), we will not only increase our understanding of the estradiol biotransport mechanism, but also benefit clinical application. Accordingly, radioactive 17β-estradiol was used to clarify the percutaneous absorption of estradiol into the 3 sublayers of the skin (stratum corneum, epidermis, and dermis) and to evaluate the effect of drugs delivered in each sublayer. Based on data thereby obtained, mathematical models were built to further obtain transport parameters (diffusivity, permeability, lag time, and partition coefficients) for the 3 layers of the skin.

Keywords: *Estradiol; human skin; transdermal transport parameters; mathematical model*

Introduction

Women experience the decline of estrogen levels during their menopausal transition between 45 and 55 years of age, as ovarian follicles show slow or no development. Symptoms include hot flushes, sweating, changes in mood, insomnia, vaginal dryness, skin atrophy, dyspareunia, and alterations of secondary sex characteristics. Severe deficiency of estrogen may lead to osteoporosis, fractures, atherosclerosis, and Alzheimer's disease (1). Oral estrogen replacement has been verified to relieve menopausal symptoms, to reverse urogential atrophy, and to decrease the risk of osteoporosis and cardiovascular diseases. Large doses of estrogen, which are needed to meet the therapeutic level during oral estrogen replacement, may result in such side effects as elevated hepatic protein levels during liver metabolic reaction and abnormal fluctuation of hormone levels in the blood. However, these undesirable effects are not observed in transdermal therapy (2). Additionally, hormone supplement therapies through transdermal absorption, a method of release that is both economical and controllable, have been accepted in research and clinics. Powers et al. (3) compared estradiol transdermal therapy with the oral method in order to understand their effects on postmenopausal women. They found that oral estrogens increased estrone to levels even beyond those observed in premenopausal women. Continuous application of transdermal estradiol over 3 weeks was not found to result in any accumulation of estradiol or estradiol conjugates. After testing the controlled release of transdermal dosage, Shaw et al. found that the ratio of circulating estradiol/estrone is around 1, whereas the estrone level for oral tests is at least 4 times higher than those for estradiol/estrone

Address for Correspondence: Prof. H. I. Maibach, Department of Dermatology, School of Medicine, University of California San Francisco, Box 0989, Surge 110, San Francisco, CA 94143-0989, USA. Tel: +1 415 476 2468; Fax: +1 415 753 5304; E-mail: maibachh@derm.ucsf.edu

(Received 12 December 2008; revised 04 June 2009; accepted 06 June 2009)

ISSN 1556-9527 print/ISSN 1556-9535 online © 2009 Informa UK Ltd
DOI: 10.3109/15569520903097622

in transdermal patch under physiological conditions (4). Akhila and Pratapkumar (5) also found transdermal hormone therapy to be significantly better than oral hormone therapy in menopausal symptom control. Patch and gel formulations presented better results in clinical efficacy and continuation rates among oral hormone therapy, percutaneous gel, and transdermal patch. Transdermal estradiol, but not oral therapy, has potential antiatherosclerotic effects as a result of the improvement of arterial stiffness (6). Oral estradiol induces antiatherogenic changes in endothelium-dependent vasodilatation and lipid concentrations (7). Compared with transdermal therapy, oral therapy changes negatively the composition of plasma lipoproteins (8).

In all such studies on the transdermal absorption of estradiol, skin was investigated as a single unit; therefore, knowledge of estradiol absorption and its properties of transport in stratum corneum, epidermis, and dermis is lacking. As a result of the combined use of mathematical models and *in vitro* human skin estradiol transport experiments, our work is an effort to explore the transport properties of the 3 structures of the skin.

Materials and methods

Radioactive 17β-estradiol was used in our research. [^{14}C] estradiol, [4-^{14}C]NEC-127 estradiol with a specific activity of 54.1 mCi/mmol, was obtained from DuPont NEN (lot number 3188-151SP; Boston, MA, USA) and was kept at 4°C until it was used. The dose was composed of estradiol 10% and vehicle 90% to include ethanal 80% and propylene glycol (PEG) 20%. Use of radioactivity was necessary to distinguish between the [^{14}C]estradiol to be absorbed through the skin and the estradiol as a natural constituent of the human body. A single dose with 15 μL/cm^2 and a radioactivity of 0.02 μC$_i$/ dose was applied on each sample. The diffusion cells are flow-through cells designed with a 1-cm^2 diffusion area and 3 mL of reservoir fluid. Incubation was conducted at 37°C and the temperature 32°C was set for skin surface. The receptor chambers were kept at 37°C throughout the experiment. Human skin samples were obtained from 4 donors by amputation, and then frozen, stored at –20°C, and thawed prior to processing. After the removal of subcutaneous fat by blunt dissection, individual portions were immersed in 60°C water for 45 seconds. At the end of each dosing period (i.e., at 0.5, 1, 2, 4, 6, and 8 hours), the dosed sites were first washed using cotton balls (Sherwood Medical, St. Louis, MO, USA), Ivory liquid soap (Proctor & Gamble, Cincinnati, OH, USA), and distilled water. The samples were taken from the cells at the end of each of the dosing periods for the separate treatments of stratum corneum, epidermis, and viable epidermis. All samples were measured using a model Tri-Carb 2900 TR (Packard Instruments, Meriden, CT, USA). Wash samples, skin sublayers, and receptor fluid were mixed directly with universal scintillation cocktail (ICN Biomedicals, Costa Mesa, CA, USA) for radioactivity assay. Tape strippings were performed after skin washing to analyze the residual dose. The samples were stripped 10 times with Scotch cellophane tape 5912 clear (3M Commercial Supply Division, St. Paul, MN, USA). These tapes were then individually placed in borosilicate glass vials with 5 mL of methanol overnight, and then 10 mL of scintillation cocktail was added for the radioactivity assay in Tri-Carb 2900 TR to measure the estradiol. The epidermis was gently stripped with a thin blade and stored in vials for radioactivity assay. The remaining dermis was also collected for further radioactivity assay.

Results and discussion

A summary of the transport and absorption results is given in Tables 1 to 6 and Figure 1. The initial dose of estradiol for each sample was 15.17 μg (100%). Estradiol absorption in the stratum corneum reached 2.83 ± 0.25 μg (18.63% ± 1.66%) after 30 minutes, peaked at 3.10 ± 0.8 μg (20.43% ± 5.29%) around 1 hour, and then gradually decreased, with the small increase of 2.84 ± 1.0 μg (18.73% ± 6.64%) at the end of the 8-hour period. For the epidermis, however, estradiol absorption peaked at the end of the 4th hour, increasing from 0.52 ± 0.09 μg (3.45% ± 0.57%) at 30 minutes to 1.16 ± 0.14 μg (7.65% ± 0.90%) at the end of the 4th hour, and to 1.13 ± 0.09 μg (7.43% ± 0.57%) at the end of the 8-hour period. Almost the same pattern existed in the case of the dermis, where absorption peaked

Table 1. Mean amount of estradiol residual in the layers of skin after 0.5 hour of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Viable epidermis (±SD)	Dermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100	71.65±7.48	18.63±1.66	3.45±0.57	0.59±0.07	0.41±0.06	94.72
Amount (μg)	15.17	10.87±1.13	2.83±0.25	0.52±0.09	0.090±0.01	0.062±0.01	14.37
Average cumulative amount of transported drug (μg)		14.37	3.5	0.67	0.15	0.06	

SD = standard deviation.

Table 2. Mean amount of estradiol residual in the layers of skin after 1 hour of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Viable epidermis (±SD)	Dermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100	54.63±10.22	20.43±5.29	6.01±1.80	4.52±1.72	1.80±0.72	87.38
Amount (μg)	15.17	8.29±1.55	3.10±0.80	0.91±0.27	0.69±0.26	0.27±0.11	13.26
Average cumulative amount of transported drug (μg)		13.26	4.97	1.87	0.96	0.27	

SD = standard deviation.

Table 3. Mean amount of estradiol residual in the layers of skin after 2 hours of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Viable epidermis (±SD)	Dermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100	37.01±8.08	7.36±5.70	4.5±0.88	8.65±0.98	39.18±3.35	96.7
Amount (μg)	15.17	5.61±1.23	1.12±0.86	0.68±0.13	1.31±0.15	5.94±0.51	14.67
Average cumulative amount of transported drug (μg)		14.67	9.06	7.94	7.26	5.94	

SD = standard deviation.

Table 4. Mean amount of estradiol residual in the layers of skin after 4 hours of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Viable epidermis (±SD)	Dermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100%	40.33±7.24	9.92±6.30	7.65±0.90	11.31±2.39	16.88±3.35	86.08
Amount (μg)	15.17	6.12±1.10	1.50±0.96	1.16±0.14	1.72±0.36	2.56±0.51	13.06
Average cumulative amount of transported drug (μg)		13.06	6.94	5.44	4.28	5.56	

SD = standard deviation.

Table 5. Mean amount of estradiol residual in the layers of skin after 6 hours of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Viable epidermis (±SD)	Dermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100%	50.85±5.57	15.58±9.22	5.57±1.65	4.73±1.06	9.27±2.04	85.99
Amount (μg)	15.17	7.71±0.84	2.36±1.40	0.84±0.25	0.72±0.16	1.41±0.31	13.04
Average cumulative amount of transported drug (μg)		13.04	5.33	2.97	2.13	1.41	

SD = standard deviation.

Table 6. Mean amount of estradiol residual in the layers of skin after 8 hours of diffusion (n = 4).

Mass balance	Dose	Surface residual (±SD)	Stratum corneum (±SD)	Epidermis (±SD)	Viable epidermis (±SD)	Reservoirfluid (±SD)	Total
Percentage (%)	100	23.64±4.50	18.73±6.64	7.43±0.57	6.20±2.15	27.75±12.93	83.75
Amount (μg)	15.17	3.59±0.68	2.84±1.00	1.13±0.09	0.94±0.32	4.21±1.96	12.70
Average cumulative amount of transported drug (μg)		12.70	9.11	6.27	5.14	4.2	

SD = standard deviation.

at 1.72 ± 0.36 μg (11.31% ± 2.39%) within 4 hours. The estradiol contained in the reservoir fluid reached its maximum of 5.94 ± 0.51 μg (39.18% ± 3.35%) at the end of the 2nd hour, then sharply decreased to 1.41 ± 0.31 μg (9.27% ± 2.04%) at the end of the 6th hour, but again quickly rose to 4.21 ± 1.96 μg (27.75% ± 12.93%) at the end of the 8th hour. The amount of cumulative transported estradiol is presented in Tables 1 to 6. For the

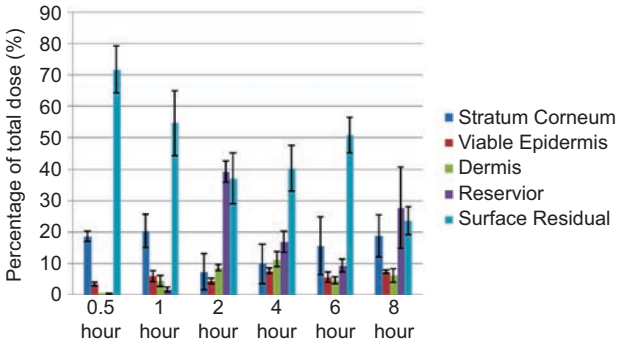

Figure 1. Mean estradiol transdermal absorption at different times (± standard deviation, n = 4).

stratum corneum, the peaks (9.06 and 9.11 μg) were reached at the end of the 2nd hour and the end of the 8th hour, respectively, with their accompanying valleys 6.94 and 5.33 μg, at the end of the 4th hour and the end of the 6th hour, respectively. The epidermis and dermis showed transport tendencies similar to that of the stratum corneum. They reached their maximums of cumulative transported drug at the end of the 2nd hour and the end of the 8th hour, respectively.

Use of the percutaneous route is, as we know, an effective approach to estrogen replacement: It helps diminish side effects resulting from conventional oral estrogen and enhances patient compliance and satisfaction because of the drug release that can be properly controlled. Our research revealed that the transport of estradiol through the skin varied significantly from stratum corneum to epidermis and to dermis ($p < .001$, power = 0.99, analysis of variance [ANOVA]). Such knowledge of the transport properties of the various layers of the skin provides us with a guide in drug administration. Up to now, however, related research is lacking, so that most researchers are still regarding the skin as a single unit in their transdermal studies of estrogen. In order to investigate the percutaneous absorption of estradiol, our study included the design of an experiment using extracted radioactive [^{14}C]estradiol to evaluate the effects of the drug delivered to the various layers of the skin: stratum corneum, viable epidermis, and dermis. By the combination of equations 1 and 2 (below), it is possible to obtain the diffusion coefficient, permeability, lag time, and partition coefficient (Table 2):

$$J_s = \frac{Dk_m C_0}{\delta} = k_p C_0 \tag{1-a}$$

$$D = \frac{\delta^2}{6\tau} \tag{1-b}$$

J_s is mass flux

where D is the diffusion constant within the skin (cm^2/h), δ is the thickness (cm) of the human stratum, τ is the lag time (h), k_p is the permeability coefficient through the skin (cm/h), k_m is the skin–vehicle partition coefficient of the drug, and C_0 is the initial concentration of the drug in the test formulation (μg/cm^3). The solution of Fick's second law (9) is as follows:

$$\frac{Q}{AC} = S[(U)t - 1/6 - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}exp(-Un^2\pi^2 t)] \tag{2}$$

t = time
n = index

where Q is the accumulative amount of flow through the membrane as measured in the experiment, A is the skin diffusion area (here 1 cm^2), and C is the given concentration in the donor cell; S and U are fitted parameters based on the experiment.

The approximate partition coefficients of skin as a complete layer were obtained:

$$K_{skin/i} = K_{sc/i}\frac{\delta_{sc}}{\delta_{skin}} + K_{ve/i}\frac{\delta_{ve}}{\delta_{skin}} + K_{de/i}\frac{\delta_{de}}{\delta_{skin}} = 2.4 \tag{3}$$

$k_{skin/i}$ = partition coefficient of skin as a complete layer
$k_{sc/i}$ = partition coefficient of stratum corneum
$k_{ve/i}$ = partition coefficient of epidermis
$k_{dermis/i}$ = partition coefficient of dermis

All calculated absorption parameters of estrogen are summarized in Table 2.

Conclusion

Currently, in most transport models for the research of estradiol transdermal absorption, the skin is regarded as a single layer, and related transport parameters have to be estimated based on the skin's average conditions.

Table 7. The percutaneous absorption parameters of estrogen.

	Stratum corneum	Epidermis	Dermis
Thickness (μm)	17	100	500
D (cm^2/hour^{-1})	1.1×10^{-7}	2.1×10^{-6}	2.0×10^{-5}
k_p (cm/hour^{-1})	1.14×10^{-3}	7.8×10^{-4}	6.4×10^{-4}
τ (hours)	4.2	8	20.8
k_m	17.6	3.7	1.6
$K_{skin/i}$		2.4	

D = diffusion constant within the skin; k_m = skin–vehicle partition coefficient of the drug; k_p = permeability coefficient through the skin; $K_{skin/i}$ = Partition coefficient of skin as a complete layer; τ = lag time.

Our research revealed that the same drug induced significantly different events in the various sublayers of the skin (i.e., stratum corneum, epidermis, and dermis), and the knowledge of such difference obviously benefits transdermal drug delivery research, an effort that will render it possible for hormone therapy to be an efficient and safe clinical practice. The mathematical model helps obtain the transport parameters and predict the transport properties for the complex structure of multiple-sublayer skin.

Acknowledgements

***Declaration of interest*:** The authors report no conflicts of interest.

References

1. Birge SJ. The use of estrogen in older women. Clin Geriatr Med 2003; 19(3):617–627, viii.
2. Jewelewicz R. New developments in topical estrogen therapy. Fertil Steril 1997; 67(1):1–12.
3. Powers MS, Schenkel L, Darley PE, Good WR, Balestra JC, Place VA. Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17 beta-estradiol: comparison with conventional oral estrogens used for hormone replacement. Am J Obstet Gynecol 1985; 152(8): 1099–1106.
4. Shaw JE, Prevo ME, Amkraut AA. Testing of controlled-release transdermal dosage forms. Product development and clinical trials. Arch Dermatol 1987; 123(11):1548–1556.
5. Akhila V, Pratapkumar. A comparison of transdermal and oral HRT for menopausal symptom control. Int J Fertil Womens Med 2006; 51(2):64–69.
6. Sumino H, Ichikawa, S., Kasama, S., Takahashi, T., Kumakura, H., Takayama, Y., Kanda, T., Kurabayashi, M. Different effects of oral conjugated estrogen and transdermal estradiol on arterial stiffness and vascular inflammatory markers in postmenopausal women. Atherosclerosis 2006; 189(2):436–442.
7. Vehkavaara S, Hokala-Ala-Pretila, T., Virkamaki, A., Bergholm, R., Ehnholm, C., Hovatta, O., Taskinen, M.R., Yki-Yarvinen, H. Differential effects of oral and transdermal estrogen replacement therapy on endothelial function in postmenopausal women. Circulation 2000; 102(22):2687–2693.
8. Vrablik M, Fait, T., Kovar, J., Poledne, R., Ceska, R. Oral but not transdermal estrogen replacement therapy changes the composition of plasma lipoproteins. Metabolism 2008; 57(8):1088–1092.
9. Crank J. Mathematics of Diffusion. Oxford, UK: Oxford University Press. 1975:47–48.