In Vivo Human Transfer of Topical Bioactive Drug between Individuals: Estradiol

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This study determined the *in vivo* human bioavailability of topical estradiol, and the transfer of drug between dosed subject and naive recipient. *In vivo* bioavailability was determined in human volunteers by ^{14}C urinary excretion following topical [^{14}C]-estradiol (0.06%) dose administration and were adjusted by intravenous human excretion amounts to give absolute bioavailability amounts. Drug transfer was determined by volunteer skin contact/rubbing for 15 minutes, 1 hour after topical dosing. [^{14}C]-estradiol bioavailability as percent dose absorbed ($n=6$) was 7.5 ± 4.1 from protected dose site, 8.2 ± 6.3 from non-protected dose site, 6.6 ± 7.6 from dosed volunteers subjected to skin contact/rub and 4.3 ± 3.8 from non-dosed volunteers subjected to skin contact/rub. Between these small groups, the values were not statistically different. Under experimental conditions, a measurable dose of radioactive 17β-estradiol dose was delivered to naive recipient volunteers through skin contact/rub with other volunteers previously topically dosed. Any residual topical bioactive chemical which resides on the open skin surface can transfer by skin contact to another individual. It is important for prescribing physicians and patients to understand that clinically significant transfer of topical bioactive drugs can occur. This may be particularly important for substances which may produce inadvertent effects.

Journal of Investigative Dermatology (2006) **126,** 2190–2193. doi:10.1038/sj.jid.5700383; published online 15 June 2006

INTRODUCTION

Topical chemical application used to be thought of as that for local drug dermatological treatment, or perhaps as a cosmetic application. Now bioactive drugs are topically administered for systemic treatment such as hormonal replacement estrogen therapy (Jewelewicz, 1997). Zondek (1938) reported that if a hormone (oestrone) ointment or hormone oil is rubbed into the shaven skin of the back of a castrated mouse the animal goes into oestrus. He also observed that the topical dose required seven times as much hormone as with subcutaneous injection. The 7× difference is topical dose not absorbed through the skin. Feldmann and Maibach (1969) showed estradiol human percutaneous absorption to be 10.6% for 24 hours dose application. The remaining dose was washed off or lost.

Studies in percutaneous absorption have shown that a portion of the topical chemical, generally a few percent of the applied dose, became systemically available. This few percent dose absorbed was sufficient for certain bioactive drugs and led to transdermal drug delivery for systemic application. During the topical drug application period, generally 24 hours, mass balance has shown that the majority of the topically applied dose was still on the skin (Wester and Maibach, 1992). In essence, an excess of drug is on the skin through the complete topical dosing period.

Johnson *et al.* (1983) showed that in 50 hospitalized patients given tetracycline ointments, creams, lotions, and tinctures containing a fluorescent marker, the topically applied medications did not remain confined to sites of initial application. Yerasi *et al.* (1997) report an unusual case where a man died of fentanyl poisoning, yet it was his wife who had the fentanyl patches. Wolf *et al.* (1997) reported childhood poisoning involving transdermal nicotine patches. Lu and Fenske (1999) report dermal transfer of pesticide residues from residential surfaces. Franklin and Geffner (2003) reported a case of pronounced virilization of a 2-year-old boy from a topical testosterone cream being used by his father. Vihtamäki *et al.* (2004) showed skin contamination by estradiol gel to be a remarkable source of error in plasma estradiol measurements. Topical estradiol and testosterone preparations are available as well as other topical bioactive preparations. This study examines 17β-estradiol human skin *in vivo* percutaneous absorption, including skin transfer between individuals.

RESULTS

Table 1 summarizes the clinical results. Urinary ^{14}C excretion was 3.9 ± 2.1, 4.2 ± 3.2, 3.4 ± 4.0, and 2.2 ± 2.0% applied

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Received 10 March 2006; revised 11 April 2006; accepted 13 April 2006; published online 15 June 2006

dose for clinical Groups A–D, respectively. These urinary excretion calculated relative to an intravenous dose (Feldmann and Maibach, 1969) gave topical bioavailability as percent doses absorbed of 7.5±4.1, 8.2±6.3, 6.6±7.6, and 4.3±3.8 for A–D, respectively. There were no statistical differences between these groups. The ^{14}C concentration in the urine samples decreased with half-lives of 28.4±9.1, 30.8±12.0, 29.3±4.5, and 33.6±13.1 hours, respectively, for Groups A–D. There were no statistical differences between these small groups.

After the 24-hour dosing period, the dosed skin site was washed with soap and water, and the skin was tape stripped on day 7 for residual chemical. The protective cover or sleeve was also analyzed for residual chemical. Table 2 shows that 73–74% applied dose was recovered in the wash from Group A after the 24-hour dose period. There was no residual chemical in day 7 tape strips. Group B wash recovery was 27–29%, and 14–24% transferred to the covering sleeve. There was no residual dose in the tape strips. Group C wash recovery was 36%, and 13% was recovered from the sleeve. Group D wash recovery was 2.3 and 4.1% recovered from the sleeve. Tape strips were not carried out on Groups C and D because the dosing area could not be defined after the skin rub/contact. Dose accountability for the groups are listed in Table 1. The results show the majority of the topical dose (Groups A and B) resides on the surface of the skin during the 24-hour dosing period. The dose is available for skin transfer (Group C) and that skin-transferred dose will be absorbed by the recipient person (Group D).

Table 1. Clinical group[1]

	Mean percent dose±SD ($n=6$)			
	A	B	C	D
Urinary ^{14}C excretion	3.9±2.1	4.2±3.2	3.4±4.0	2.2±2.0
Topical bioavailability[2,3]	7.5±4.1	8.2±6.3	6.6±7.6	4.3±3.8
$T_{1/2}$ renal excretion (hours)	28.4±9.1	30.8±12.0	29.3±4.5	33.6±13.1
Dose accountability[4]	82.0±5.3	56.9±11.0	56.9±7.1	10.8±7.9

[1]A=dose site protected with non-occlusive cover (n=6).
B=Dose site not protected (n=6).
C=skin site dosed, then rubbed on volunteer in group D (n=6).
D=naïve recipient of skin contract/rub (n=6).
[2]Topical bioavailability=urinary excretion (topical dose)/urinary excretion (i.v. dose)×100; 51.6% dose excreted after single i.v. dose human (Jewelewicz, 1997).
[3]No statistical difference ($P>0.05$).
[4]Please note that mass balance is higher where topical dose was better controlled.

DISCUSSION

Topically applied chemicals reside on the skin for an extended dosing period, and these chemicals can transfer through skin contact with another naive person. This is probably of no consequence for inert chemicals; however, potent topical bioactive chemicals have become available. An example given previously was of topical testosterone on a father which this study has shown could transfer by contact to his son. The testosterone cream was applied without attention to hand washing after application or the possibility of contact transmission to other family members. This began a few months after his son's birth (Franklin and Geffner, 2003). Different from testosterone which may induce inadvertent effects in immature and female individuals, no relevant risk scenario is currently known from possible transfer of 17β-estradiol.

Our study, using single defined doses of labeled estradiol (about 0.1 mg) and demonstrating absorption rates <10%, was unlikely to produce systemic biological effects in this case. Topical drugs intended for systemic delivery are routinely used over an extended period of time. Our Group A results show that 73–74% protected estradiol dose was still on the skin, and Group B showed 27–29% dose still on the unprotected skin and 14–24% on the clothing (sleeve), probably all still available for absorption. Since the total bioavailability was 7.5% for Group A and 8.2% for Group B, a large portion of active substance is still available for transfer/absorption. This study does not answer the question of how long residual topical drug would be available for

Table 2. Clinical group – treatment forearm

		Mean percent dose±SD ($n=6$)			
		A	B	C	D[1]
Wash	Left	73.0±10.0	27.2±4.0	36.1±14.4	2.3±2.0
24 hours	Right	74.0±6.1	29.0±4.0	—	—
Cover[2]	Left	0.9±6.1[2]	24.2±14.4[3]	12.9±6.6[3]	4.1±3.6[3]
or					
Sleeve[3]	Right	1.2±1.6[2]	13.7±3.8[3]	—	—
Tape strip	Left	0.02±0.01	0.05±0.07	—	—
Day 7	Right	0.02±0.02	0.03±0.03	—	—

[1]The percent recovery in Group D is based on the dose applied to Group C.
[2]Cover is non-occlusive/non-skin touching cover over the dosed site.
[3]Sleeve is disposable cover placed over the arm to stop the spread of radioactivity to the volunteer's clothing and environment.

transfer relative to time on skin, formulation changes, or effect of routine skin washing.

Topical bioavailability using cumulative urinary recovery of estradiol in this study was 7.5 ± 4.1 and $8.2 \pm 6.3\%$ doses for Groups A and B. This compares favorably with the published *in vivo* estradiol percutaneous absorption of $10.6 \pm 4.9\%$ doses (Feldmann and Maibach, 1969). This study and the report by Franklin and Geffner (2003) document transfer of estradiol and testosterone. During discussions on sport's doping (BALCO, Olympics), the ''cream'' (testosterone) gets mentioned as a contributing factor. There is the report cited earlier of suspected fentanyl transfer (Yerasi *et al.*, 1997). This transfer potential will apply to any bioactive topical chemical, and this should not be restricted to drugs. Hazardous environmental chemicals (pesticides, herbicides, pollutants) can topically settle on one individual and be transferred to another person.

This study shows only the one rub/contact sequence for one time period after dosing for one formulation. In the real world, skin contact will occur under a variety of conditions, and each variable will contribute to overall chemical skin transfer. More data are thus warranted. However, the potential for skin transfer of topical bioactive chemicals has been shown *in vivo* in human volunteers and an awareness of this effect should be heeded.

MATERIALS AND METHODS

Dose formulation

[^{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), and was kept at 0–4°C until used. Radiochemical purity of 98.5% was determined by HPLC and thin layer chromatography.

The formulation was a gel (oestrogel) prepared according to the manufacturer. An appropriate amount of [^{14}C]-estradiol was incorporated to be a 0.06% (w/w) dose formulation containing 5 mg [^{14}C]-estradiol radioactivity per 8.5 g of gel (131 μCi/g). The radioactive estradiol was incorporated into the gel. Triplicate aliquots were assayed to determine uniformity of formulation. Use of radioactivity was necessary to distinguish between that estradiol [^{14}C] which was absorbed through the skin and that estradiol which is a natural constituent of the human body.

Study design

Group A was to determine topical absorption of [^{14}C]-estradiol under a protective condition during a 24-hour exposure period. The protective non-occlusive cover placed on the dosed area and kept for 24 hours was to protect the dosed site and exclude drug loss. After 24 hours, the dosed site was washed with soap and water and the washing sample was retained for ^{14}C assay. The cover was assayed for possible residual chemical.

Group B was to determine topical absorption on [^{14}C]-estradiol under normal use conditions, which had no protective device during the 24-hour exposure period.

Group C was to determine how much [^{14}C]-estradiol transferred after contact with Group D. At 1 hour after topical dose, the dose area was rubbed and contacted by a Group D volunteer who did not receive any dose.

To prevent [^{14}C]-estradiol transferring from the dosed site skin to other skin area via contaminated clothes, a Tyvek paper sleeve (Lab Safety Supply) was placed on each dosed forearm and kept in place for the 24-hour dosing period for Groups B–D volunteers. After 24 hours, the dosed site was washed with soap and water, and the washings retained for ^{14}C assay. The sleeve was assayed for possible residual chemical.

The experimental design and performance was followed the Declaration of Helsinki Principles (Ethical Principles for Medical Research Involving Human Subjects, see: www.wma.net) and approved by the Committee on Human Research at the University of California, San Francisco. Human volunteers were recruited from the University of California, San Francisco and surrounding San Francisco Bay Area community. Each volunteer signed a Consent Form before the study. Eighteen healthy postmenopausal women aged from 42 to 78 years were selected for Groups A–C. Six normal, healthy men or women aged from 29 to 76 years were selected from Group D. Each group consisted of six volunteers.

Dosing procedure

Group A. A 50 cm^2 area (3.5 × 14.3 cm^2) was marked on the left and right ventral forearms of each volunteer. The marked areas received a single topical application on each forearm of total 0.17 g (22 μCi) [^{14}C]-estradiol gel formulation delivered with a 0.1 ml Teflon-coated syringe (Hamilton Company, Reno, NV). The delivered dose was quantitated by weighing the microsyringe before and after dosing. This was carried out for all dosings. After topical applications, a non-occlusive solid plastic cover was secured over each dosed area of Group A volunteers and kept in place by tape for 24 hours. The cover was made by cutting a plastic cylinder (20 cm length × 5 cm diameter) in half lengthwise, and drilling three 1 cm diameter holes in the surface. This allowed free movement of air from its two open holes on the top. The volunteers were requested not to touch or wash the dosed area for 24 hours (this was asked of all groups).

Group B. A 50 cm^2 area (3.5 × 14.3 cm^2) was marked on the left and right ventral forearm of each volunteer. The marked areas received a single topical application in each forearm of total 0.17 g (22 μCi) [^{14}C]-estradiol gel formation delivered with a 0.1 ml Teflon-coated syringe (Hamilton Company, Reno, NV). After dosing, the dosed area was allowed to air dry for 1 hour after topical application. After 1 hour, a Tyvek paper sleeve was placed on each dosed forearm for 24 hours.

Group C. A 100 cm^2 area (5.0 × 20 cm^2) was marked on the left ventral forearm of Group C volunteers. The marked area received a single topical application of 0.16 g (22 μCi) [^{14}C]-estradiol gel formulation delivered with a 0.25 ml Teflon-coated syringe (Hamilton Company, Reno, NV).

After topical application, the dosed area was allowed to air dry for 1 hour, then rub and contact the ventral forearm (containing drug) on the ventral forearm of Group D volunteers for 15 minutes. After rubbing, the dosed forearm of Group C volunteers was placed in a sleeve for 24 hours. The volunteers were requested not to touch or wash the dosed area for 24 hours.

Group D. A ventral forearm of Group D volunteers was in contact with the ventral forearm (containing drug) of Group C volunteers. Skin contact consisted of rubbing five times up and down in one direction (parallel to the axis of the arm), then close contact of skin

surfaces for 15 minutes, then rubbing five times up and down in the other direction. After rubbing, the contacted forearm of Group D was placed in a sleeve for 24 hours.

Skin washing and analysis

At 20 hours after dosing, the cover or sleeve was removed and each dosed site was washed using cotton balls (Sherwood Medical, St Louis, MO), Ivory liquid soap (Proctor & Gamble, Cincinnati, OH) and water. The water washing procedure was as follows:

(1) 50% Ivory soap solution (v/v).
(2) Distilled deionized water.
(3) 50% Ivory soap solution (v/v).
(4) Distilled deionized water.
(5) Distilled deionized water.

Each skin dosed area of Groups A–C volunteers was washed five times. The area outside of the dosed skin on the ventral forearm (called ''non-dosed skin site'') of Group C was also washed. Since the whole skin area on the ventral forearm of Group D volunteers was considered to receive the topical formulation after rubbing, it was divided into two parts, the middle-upper and middle-lower of the ventral forearm. Each part was washed five times using the washing procedure. Individual cotton balls were placed in a borosilicate glass vial containing 5.0 ml of methanol overnight and then 10.0 ml scintillation cocktail was added. Appropriate dilutions were made based on the radioactivity. Radioactivity in these samples was analyzed by liquid scintillation counting to quantify the amount of [^{14}C]-estradiol removed from the application site. The cover was also washed five times using the above procedure. The cotton balls were individually placed in a borosilicate glass vial with 10.0 ml scintillation cocktail. Radioactivity of these samples was counted using the liquid scintillation counting.

Skin tape stripping and analysis

The tape stripping on Groups A and B was carried out 168 hours after skin washing to analyze the residual dose. The dosed skin site was stripped with Scotch® cellophane tape 5912 clear (3 M Commercial Supply Division, St Paul, MN) 10 times. These tapes were then individually placed in borosilicate glass vials with 5 ml of methanol overnight, then 10 ml of scintillation cocktail was added, and subsequently assayed for radioactivity by liquid scintillation counting. Tape stripping was not carried out on Groups C and D because the skin rubbing sequence changed the original dosing area.

Radioactivity of sleeve

The sleeve was cut into 20 pieces and individually placed in borosilicate glass vials with 5 ml of methanol overnight, then 10 ml of scintillation cocktail was added, and subsequently assayed for radioactivity by liquid scintillation counting.

Urine sample collection and analysis

Urine samples were collected 0–24, 24–48, 48–72, 72–96, 96–120, 120–144, and 144–168 hours after dosing. The volume of urine sample was measured and aliquots of urine sample (approximately 20 ml) were kept. Duplicate aliquots of 1.0 ml were used to determine radioactivity. Estradiol topical bioavailability was determined by urinary ^{14}C excretion and calculated relative to an estradiol human intravenous dose (Feldmann and Maibach, 1969) to determine absolute bioavailability.

Radioactivity assay

All radioactivity measurements were conducted using a Model 1500 Liquid Scintillation Counter (Packard Instruments, Downers Grove, IL). The counter was audited for accuracy using sealed samples of quenched and unquenched standards as detailed by the instrument manual. Background control and test samples were counted in duplicate where possible for 3 or 5 minutes each. Weighed aliquots or urine, skin wash, tape strip, sleeve, and cover wash samples, skin, receptor fluid were mixed directly with Universal Scintillation Cocktail (ICN Biomedicals, Costa Mesa, CA) and analyzed for radioactivity.

Statistical analysis and topical bioavailability

Statistical analysis (*t*-test, one way analysis of variance) was carried out using SIGMASTAT (SPSS Inc., Chicago, IL). Topical bioavailability was calculated as urinary excretion (topical dose)/urinary excretion (i.v. dose) × 100. The data of urinary excretion following a single i.v. dose, 51.6% dose excretion, was cited from Feldmann and Maibach (1969) (Jewelewicz, 1997).

CONFLICT OF INTEREST

UCSF contracted with Dr Kade (Dr Kade Pharmazeutische Fabrik GMBH, Berlin, Germany) for this study. No other funds or conflict of interest occurred.

ROLE OF THE FUNDING SOURCE

Dr Kade (Dr Kade Pharmazeutische Fabrik GMBH, Berlin, Germany) provided the funds and instruction on preparation of the gel. Otherwise, sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

ACKNOWLEDGMENTS

We thank Dr Kade for the funds to do this study, and Nick Poblete for excellent laboratory and technical assistance.

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