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Percutaneous absorption of hydrocortisone and testosterone on the vulva and forearm: effect of the menopause and site

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Summary The percutaneous absorption of hydrocortisone and testosterone was studied following their application to the vulvar and ventral forearm regions of pre- and post-menopausal women. Percutaneous absorption of hydrocortisone was significantly greater in vulvar skin than forearm skin in both pre- and post-menopausal women ($P<0{\cdot}05$, respectively), whereas the percutaneous absorption of testosterone was significantly increased ($P<0{\cdot}01$) on the vulva compared with the arm only in post-menopausal women.

The effect of age on the percutaneous absorption of hydrocortisone and testosterone was evaluated by using the menopause as a biological chronometric end point. It is a common misconception that older skin has a diminished barrier capacity, and that percutaneous absorption is therefore greater. Our studies showed that absorption of hydrocortisone vulval skin of pre-menopausal women was significantly greater ($P<0{\cdot}01$) than in post-menopausal women. The ventral forearm skin of pre-menopausal women tended to show increased absorption compared with post-menopausal women, but statistical significance was not reached. No significant differences ($P>0{\cdot}05$) in the percutaneous absorption of testosterone in vulval or forearm skin were observed between the two age groups.

The skin plays a vital role in maintaining internal homeostasis by regulating the loss of water and electrolytes, and protecting the body against the ingress of harmful substances. A principal regulatory barrier of the epidermis is believed to be located in the stratum corneum.[1] The stratum corneum is a selective membrane which allows certain substances to pass more readily, and there are regional differences in percutaneous absorption. Anatomical sites of increased permeability have been used in transdermal drug delivery systems. For example, scopolamine and testosterone patches were applied to preauricular and scrotal areas, respectively, for maximum absorption.

The menopause, a significant physiological marker of ageing, may provide a useful chronological parameter in the evaluation of the effects of cutaneous ageing in women. The present study investigated the influence of site and age (as reflected by hormonal status) on the percutaneous absorption of hydrocortisone and testosterone.

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Methods

Materials and subjects

^{14}C-labelled hydrocortisone and testosterone (New England Nuclear, Boston, MA) were applied either to the ventral forearm or labia major of healthy, non-hirsute, pre-menopausal (age 21–35 years) and post-menopausal (48–72 years) women ($n \geqslant 9$ for each compound, site and age group). The average age of the pre-menopausal women was 32 years, and that of the post-menopausal women was 55 years. This study was approved by the UCSF Committee on Human Research and the Radioactive Safety Committee. Informed written consent was obtained from the participants before they were enrolled in the study. All pre-menopausal women were required to have negative pregnancy tests. All subjects were asked not to take oral contraceptives or supplement hormones. The study was performed during the months of March to May.

Application conditions

The chemical and radioactive doses were 4 μg/cm^2 and no more than 1·0 μCi/cm^2, respectively. The steroids were applied from a 20 μl acetone solution on to an

area of 2·5 cm^2, which was outlined with a stencil. Application was performed by using a glass microsyringe, and the solution was spread evenly over the site. The sites of application were: (i) the ventral surface of the forearm, 5 cm from the antecubital fold, and (ii) the midlabium majus, approximately 1 cm lateral to the vaginal orifice. Hair was trimmed from each site with electrical scissors. The protective, non-occlusive chamber described by Bucks *et al.*[2] was employed to cover the sites, and was placed on the skin 30 s after application of the solution. The chamber was secured to the arm or vulva, and the subjects were asked not to shower or bathe, and to avoid vigorous exercise. Following the 24 h application period, the chamber was removed and the site washed with soap and water, using a standardized washing procedure.[3] A second patch was applied to protect the site of application from friction, and remained in place until day 7, when the washing procedure was repeated. After the washed site was air-dried, it was cellophane tape-stripped 10 times using a new piece of tape for each stripping procedure.

Percutaneous absorption measurements

Subjects collected their urine for 7 days at intervals of 0–4, 4–8, 8–12, 12–24, 24–36, 36–48, 48–60 and 60–72 h, and then every 24 h. Total urine volume was measured for each time interval. Duplicate 5 ml aliquots were analysed for radioactivity by mixing with 10 ml of scintillation cocktail (Beckman Ready Gel Scintillation Cocktail, Fullerton, CA) and counting in a liquid scintillation counter. Absorption data were corrected for incomplete renal elimination by using an intravenous correction factor.[4]

Measurement of chemical retention within the stratum corneum

The long-term reservoir (substantivity) of hydrocortisone and testosterone in the stratum corneum of the vulva and the ventral forearm was measured by analysis of the cellophane tape strips collected on day 7. Each tape strip was placed in 10 ml of scintillation cocktail and, after 48 h, radioactivity was assayed by liquid scintillation counting.

Statistical analysis

Statistical comparisons were performed using one way analysis of variance followed by the Neuman–Keuls multiple range tests.[5]

Results

The effects of anatomical site and age on the percutaneous absorption of hydrocortisone and testosterone, following a 24 h application, are shown in Figure 1. Each plot represents the absorption of the radiolabel overtime, plotted at the midpoint of the collection interval (with correction using the fraction of an intravenous dose eliminated in the urine).

The cumulative percutaneous absorption of hydrocortisone and testosterone are summarized in Table 1. Cumulative absorption of hydrocortisone was significantly higher on the vulva than the arm in both pre- and post-menopausal women ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively). There was a general trend for increased testosterone absorption on the vulva compared with the arm, but the difference was statistically significant only in post-menopausal women ($P < 0{\cdot}01$). Cumulative absorption of hydrocortisone was greater on vulvar and forearm skin in pre-menopausal women than post-menopausal women. The cumulative absorption of hydrocortisone was significantly greater ($P < 0{\cdot}01$) on the vulva of pre-menopausal than post-menopausal women. Testosterone cumulative absorption tended to be higher in pre-menopausal women. Testosterone cumulative absorption tended to be higher in pre-menopausal women; however, no statistical significance was observed, and the kinetics of absorption were similar.

Long-term reservoir (substantivity) or hydrocortisone in the stratum corneum of the vulva and the ventral forearm of both pre- and post-menopausal women was calculated from analysis of the tape strips obtained on day 7, following soap and water washing. For the arm, hydrocortisone levels in pre- and post-menopausal women were $2{\cdot}5 \pm 1{\cdot}2$ and $1{\cdot}9 \pm 1{\cdot}3$, respectively, whereas, vulvar levels were $0{\cdot}74 \pm 0{\cdot}45$ and $1{\cdot}1 \pm 0{\cdot}3$ in pre- and post-menopausal women, respectively. Stratum corneum levels of hydrocortisone on the arm were significantly ($P < 0{\cdot}05$) higher than on the vulva of pre-menopausal women. Hydrocortisone distribution was not limited to the upper layers of the stratum corneum (Fig. 2). Testosterone was not detected in the tape strips.

Discussion

Our results confirm earlier work by Britz *et al.*[6] that vulvar skin shows increased percutaneous absorption of hydrocortisone. However, these authors did not investigate the effect of age, as reflected by the hormonal

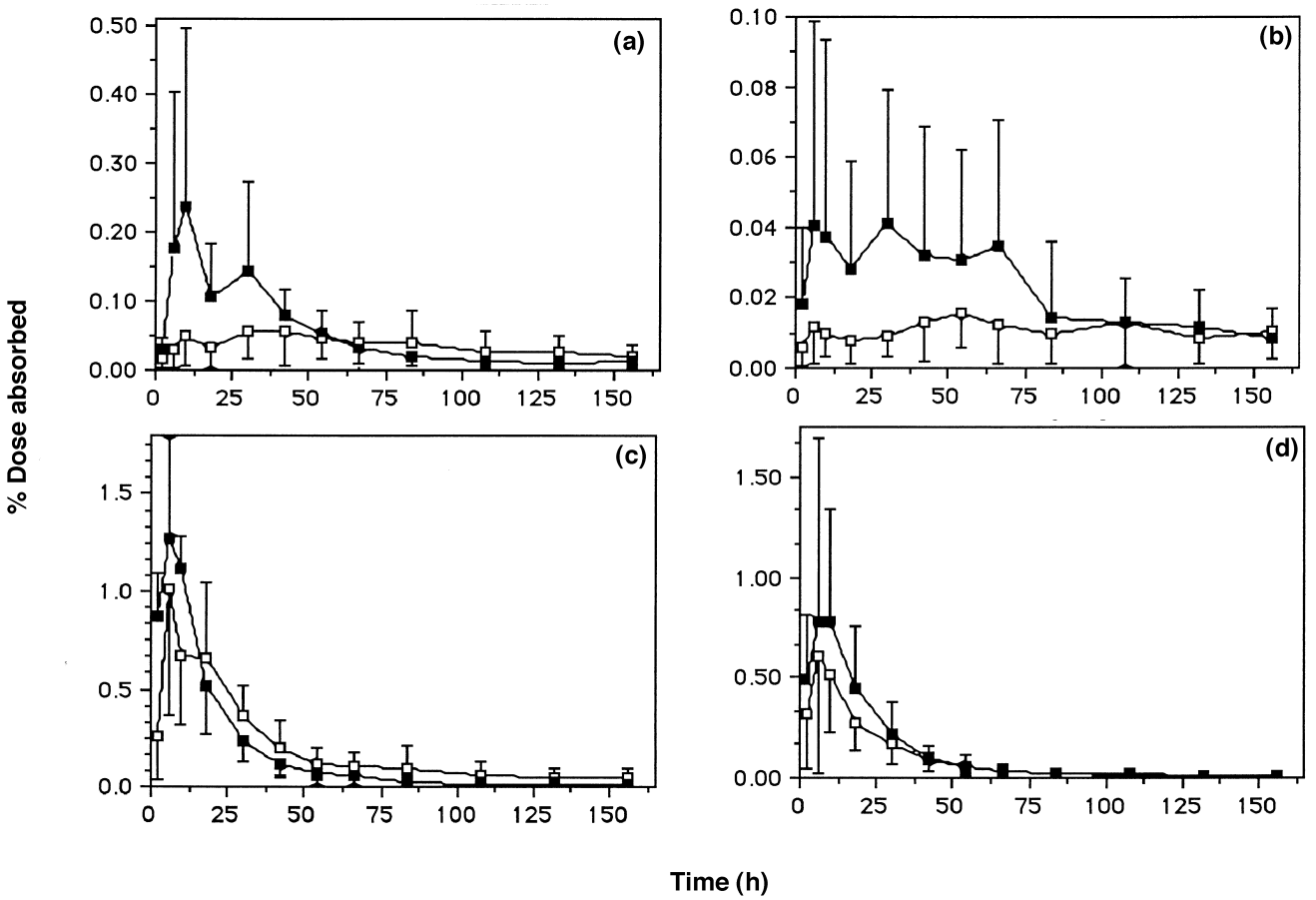

Figure 1. Effect of age on the percutaneous absorption of hydrocortisone and testosterone. (a) and (b) show hydrocortisone absorption on the vulva and forearm, respectively. (c) and (d) show testosterone absorption on the vulva and forearm, respectively. Each point represents the mean ± (standard deviation) (SD) of at least 10 determinations. (□), post-menopausal women; (■), pre-menopausal women.

Table 1. Cumulative percutaneous absorption of steroids in pre-menopausal (PRE) and post-menopausal (POST) women following a single 24 h exposure

Compound	Site	Age	% Absorbed (mean ± SD)
Hydrocortisone	Arm	Pre	2·8 ± 2·4
		Post	1·6 ± 1·2
	Vulva	Pre	8·1 ± 4·1
		Post	4·4 ± 2·8
Testosterone	Arm	Pre	20·2 ± 8·1
		Post	14·7 ± 4·2
	Vulva	Pre	25·2 ± 6·8
		Post	24·3 ± 5·4

a, $P < 0{\cdot}01$; b, $P < 0{\cdot}05$; c, not significant, using one-way analysis of variance followed by the Neuman–Keuls multiple range test.
SD, standard deviation.

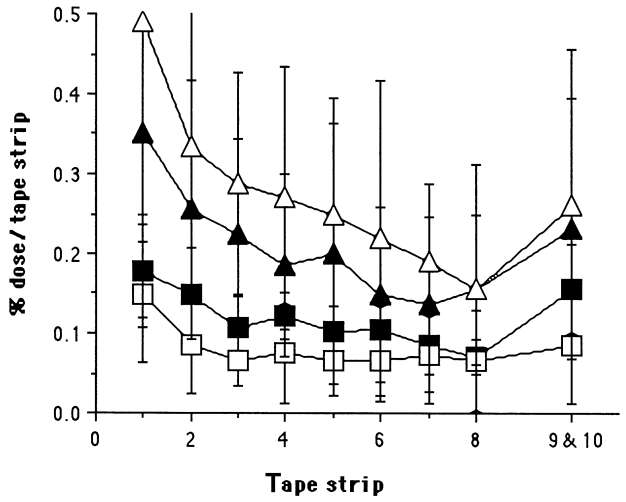

Figure 2. Hydrocortisone in the stratum corneum of the vulva and forearm of pre- and post-menopausal women, on day 7. Stratum corneum removed by 10 consecutive cellophane tape strips, following soap and water washing. Each point represents the mean ± standard deviation (SD) of at least seven determinations. (–■–), vulva (post-menopausal); (–□–) vulva (pre-menopausal); (–▲–), arm (post-menopausal); (–△–), arm (pre-menopausal).

status. When the percutaneous absorption of testosterone was compared at the two sites, only the vulva in post-menopausal women, showed increased absorption. Hence, vulvar skin exhibits increased permeability to selected compounds.

The skin, like any other organ system, undergoes changes with the passage of time. The absorption of hydrocortisone was less in post-menopausal than pre-menopausal skin. These findings are in agreement with the work of Christophers and Kligman,[7] Rougier *et al.*[8] and Roskos *et al.*,[9] who also observed decreased absorption of different compounds in the older age group. However, we did note a statistical difference on ventral forearm skin. As the vulvar skin is under hormonal influence, the differential absorption of hydrocortisone may be explained by the onset of the menopause. To date, we are not aware of any study demonstrating an increase in absorption in older age groups, despite the common belief that older skin has diminished barrier function. The percutaneous penetration of testosterone was not significantly different between the two age groups.

In general, there is greater cutaneous permeability to testosterone than to hydrocortisone. This difference may be related to hydrocortisone's greater hydrophilic properties. The permeability to both hydrocortisone and testosterone is greater on the vulva than the forearm. This difference in the bioavailability of a compound according to site may be related to stratum corneum thickness or appendages (e.g. hair follicles or sweat ducts). For example, the absence of apocrine glands on the forearm may account for the difference. Finally, vulvar cutaneous blood flow may contribute to increased permeability. However, we believe the latter is of minor importance.

Tropical testosterone is an effective therapy for lichen sclerosus et atrophicus.[10,11] Of concern are the potential side-effects of testosterone, because treatment often requires many months of application. Although our studies do not correlate therapeutic efficacy with the amount of testosterone penetration, it is apparent that testosterone absorption is not significantly different between pre- and post-menopausal women, when comparing absorption at the same site. Assuming similar target organ response in both age groups, the virilizing potential of topical testosterone application would be similar.

Substantial levels of hydrocortisone, but not testosterone, were present in the stratum corneum on day 7. This was resistant to removal by soap and water washing, and was not localized in the upper layers of the stratum corneum. These results suggest that age (as reflected by the hormonal status), in addition to site, affects long-term substantivity. In general, the forearm skin was observed to retain greater levels of hydrocortisone and to be less permeable to hydrocortisone than the vulva.

In summary, steroids were topically applied to the vulva and the ventral forearm of pre- and post-menopausal women. In both groups, the cumulative percutaneous absorption of hydrocortisone was greater on the vulva than on the forearm, and on the vulva it was higher in pre-menopausal than post-menopausal women. For testosterone, however, the greater cumulative absorption on the vulva was seen only in post-menopausal women, and there were no differences between age groups. The age-related (as reflected by the hormonal status) and site-related differences in the absorption and long-term retention of hydrocortisone (and possibly other compounds) may have potential ramifications in the prescribing of drug therapy for an elderly population.

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