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Interpersonal testosterone transfer after topical application of a newly developed testosterone gel preparation

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Summary

OBJECTIVE Transdermal testosterone gel treatment is an effective androgen substitution therapy with several advantages over conventional substitution therapies. Whereas side-effects due to overdosing of hypogonadal patients are unlikely, testosterone gel application without protection may cause severe side-effects in other subjects (partners, family members) by contamination. Therefore, the risk of testosterone transfer of a newly developed 2·5% testosterone gel preparation was evaluated.

DESIGN In two clinical randomized open single-centre studies on healthy male volunteers the percentage of testosterone remaining on the skin after gel application over time (n = 12) and the possibility of a transfer of testosterone to another person (n = 28) was evaluated. In the second study the endogenous testosterone production in the receiving subjects was suppressed by injecting 400 mg norethisterone enanthate (NETE).

RESULTS After 8 h approximately 60% of testosterone applied to the skin could be recovered. When the skin had been previously washed with water, only about 14% of applied testosterone could be recovered. After intense skin contact with a volunteer who had applied testosterone before on his forearm, no increase in testosterone serum levels could be found in NETE-suppressed men.

CONCLUSION Although considerable amounts of testosterone remain on the intact skin for several hours after evaporation of the alcohol vehicle, contamination of a second, especially female or prepubertal, subject causing side-effects seems very unlikely.

Testosterone substitution therapy is recommended for men with congenital or acquired primary and secondary hypogonadism. Several preparations are available for intramuscular, oral or transdermal application. Testosterone enanthate and testosterone cypionate are the preparations most widely used for intramuscular injection, while testosterone undecanoate is available for oral application. Nonetheless, the pharmacokinetic properties of these compounds are far from ideal. They all produce marked fluctuations in serum testosterone levels with unphysiologically high levels after administration (Nieschlag & Behre, 2000).

Transdermal testosterone offers advantages over oral and intramuscular application of testosterone: both the first-pass metabolism in the liver after oral application, requiring high testosterone doses, and potentially painful injections combined with supraphysiological testosterone serum concentrations are avoided. Moreover, the endogenous circadian rhythm of testosterone secretion can be imitated. However, transdermal testosterone is also characterized by certain drawbacks. After receiving the non-scrotal patch, 7% of patients developed allergic contact dermatitis at the site of patch application and moderate irritation was noted in 32% of patients (Jordan, 1997). After application of scrotal testosterone patches, supraphysiological dihydrotestosterone (DHT) serum levels were measured (Bals-Pratsch *et al*., 1986; Behre *et al*., 1999; Cunningham *et al.*, 1989). Moreover, good adhesion of the patch requires shaving of the scrotal skin. For these reasons improved testosterone preparations would be desirable. Recently, a testosterone gel (AndroGel®; Unimed Pharmaceuticals Inc., 2000) became commercially available in the USA. With this testosterone gel containing 1% testosterone, physiological serum levels can be maintained for more than 24 h. Replacement using testosterone gel improved sexual function and mood, increased lean mass and muscle strength, and decreased fat mass in hypogonadal men with less skin irritation and discontinuation compared with the recommended dose of the permeation-enhanced Androderm® patch (Swerdloff *et al*., 2000).

Topical application of androgens without protection may cause side-effects in other persons due to contamination, especially in prepubertal children and females (Delanoe *et al*., 1984; Schaison & Couzinet, 1998). In general, androgens administered

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to prepubertal children of both sexes have the capacity to promote premature epiphyseal closure and induce premature virilizing action; in women testosterone administration leads to hirsutism, acne, coarsening of the voice, clitoris hypertrophy and male pattern baldness (Rolf & Nieschlag, 1998). These side-effects are largely irreversible when androgen treatment is discontinued.

For the development of a new testosterone gel preparation we performed two studies to evaluate the risk of unplanned contamination of a partner. Previously we had demonstrated that the testosterone gel preparation is able to penetrate through the skin, resulting in elevated testosterone serum levels (Rolf *et al*., 2002).

Subjects and methods

Subjects

Both studies were single-centre, open, randomized studies. The protocols were approved by the Ethics Committee of the University of Münster and the State Medical Board. The studies were conducted according to good clinical practice (GCP). For study I, 12 healthy male volunteers (aged 22–27 years) were recruited, and for study II, 28 healthy male volunteers (aged 21–37 years) were recruited. None of the volunteers suffered from acute or chronic skin diseases. No concomitant medications were allowed during the studies and all subjects denied their use during the course of the study. Prior to enrolment, each subject's written informed consent was obtained in response to a fully written and verbal explanation of the nature of the study. A thorough medical history was taken, followed by a physical examination and routine clinical chemistry and haematology in study II.

Study I

At the beginning of the study, the skin was washed thoroughly, and 2×6 areas of 80 cm^2 each on both sides of the abdomen and 2×1 area on the inner sides of both forearms were marked with a black text marker. The newly developed 2·5% testosterone gel (0·4 g) was applied to each of the 14 skin test areas according to a random schedule. The gel was applied by plastic syringe; the exact amount of gel was calculated by subtracting the weight of the syringe after gel application from that before application. After gel application the skin area remained uncovered.

After 10 and 30 min, and after 1, 2, 4 and 8 h, the remaining testosterone was extracted successively from the marked abdominal areas in random order with an alcohol-containing cotton-wool swab for 1 min each. On the left side the skin was washed thoroughly with a wet single-use washrag 5 min prior to gel extraction with alcohol.

To evaluate the possible transfer of the gel, 30 min after gel application onto the left inner side of the forearm, the forearm was rubbed with the corresponding right arm for 5 min. Afterwards, the remaining testosterone and the transferred testosterone, respectively, were extracted with an alcohol-containing cotton-wool swab for 1 min. The cotton-wool swabs were stored in sealed glass vials until determination of testosterone.

Study II

In study II 14 volunteers (group I) were injected with 400 mg noresthisterone enanthate (NETE, Schering AG, Berlin, Germany) to suppress endogenous testosterone production and to minimize testosterone fluctuations, as described by Kamischke *et al*. (2000). On the fifth day after injection of group I the other 14 volunteers (group II) applied 5 g of the newly developed 2·5% testosterone gel onto the inner side of their forearms to an area of approximately 300 cm^2. After 10 min, when the alcohol had evaporated, half of the volunteers in group II washed their arms with soap and water, and then dried them thoroughly. Directly afterwards, all donors rubbed their arms against the back of the testosterone-suppressed recipients for 10 min. Serum samples for testosterone determination were taken from the recipients before and directly after skin contact and 20 and 110 min after skin contact. On day 6 after NETE administration an additional 24-h profile was performed to evaluate the fluctuation of testosterone serum levels.

Thereafter, volunteers of group II were injected with 400 mg NETE and the study was repeated as described above, whereby this time the volunteers of group I served as donors but applied only 2·5 mg of the 2·5% testosterone gel to their forearms.

Hormone determinations

For study I, testosterone was extracted from the swabs with 100 ml 2-propanol solution, and 10 µl were immediately measured by validated reverse-phase high-performance liquid chromatography and UV-detection (German Pharmacopoeia, 1990). The detection limit was 0·0492 mg/100 ml. The intra- and interassay coefficients of variation were 2·6% and 1·5%, respectively.

For study II testosterone was measured in serum samples by a commercial direct solid-phase enzyme immunoassay (DRG AURICA ELISA Testosterone Kit; DRG Instruments GmbH, Marburg, Germany). The detection limit was 0·469 nmol/l. The intra- and interassay coefficients of variation were 3·7% and 4·8%, respectively.

Statistical analysis

Results are reported as the mean ± SD. Comparisons between the testosterone values of the individual time-points were performed by ANOVAs on ranks for repeated measures. Computations were performed using the statistical software package SigmaStat

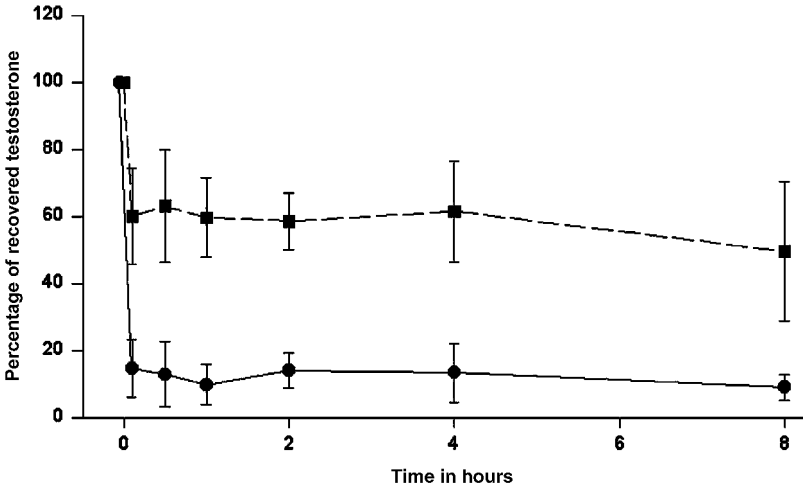

Fig. 1 Amount of testosterone (as percentage of total amount applied; mean ± SD) that could be recovered from the skin with alcohol after application of 0·4 g of a 2·5% testosterone gel formulation. The total amount of testosterone applied was 11·0 ± 0·7 mg. ●, with washing prior to testosterone recovery; ■, without washing prior to testosterone recovery.

2·03 statistical software for Windows (Jandel Scientific GmbH, Erkrath, Germany).

Results

Study I

In none of the samples taken before gel application could testosterone be detected. A mean amount of 11·0 ± 1·7 mg testosterone was applied to each skin area. Ten minutes after gel application approximately 60·2% of the applied testosterone (6·8 ± 1·8 mg) could be recovered with alcohol from the unwashed skin areas but only 14·7% (1·8 ± 1·3 mg) from the previously washed skin areas. No significant further decline was measured in the following hours. After 8 h a decline in the amount of testosterone to 49·7% (5·0 ± 2·0 mg) was observed from the unwashed skin areas; from the previously washed skin areas 9·1% (1·4 ± 1·5 mg) could be extracted after 8 h (Fig. 1).

A mean amount of 11·7 ± 2·2 mg testosterone was applied to each skin area of the right forearm. After skin contact for 5 min, 3·1 ± 1·8% of the applied testosterone was recovered from the corresponding skin area of the left arm.

Study II

In the groups of recipients who had received norethisterone injections, clearly suppressed testosterone serum levels were observed before skin contact (group I, 3·2 ± 1·3 nmol/l; group II, 2·6 ± 0·7 nmol/l). Directly after skin contact a short-term increase in testosterone serum levels up to 9·2 nmol/l was measured in only one volunteer in group II; this brief elevation was probably caused by contamination of the indwelling catheter, as an increase of more than 6 nmol after 10 min of indirect contact with the test drug is impossible. This subject was therefore excluded from evaluation.

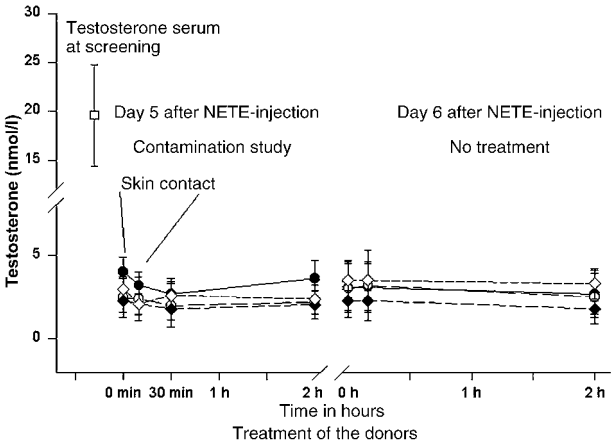

Fig. 2 Testosterone serum levels (mean ± SD) in testosterone-suppressed recipients after thorough skin contact for 10 min with donor who had previously applied 5·0 or 2·5 g of a 2·5% testosterone gel on their forearms. Treatment of the donors: ●, group 1a (5 g testosterone, with washing); ○, group 1b (5 g testosterone, without washing); ◆, group 1a (2.5 g testosterone, with washing); ◇, group 1b (2.5 g testosterone, without washing).

No increase in testosterone serum levels was observed either in the groups of donors who washed their skin before or in the groups of donors who did not wash their skin after gel application. No significant differences in testosterone serum levels were found compared with those observed without treatment the following day (Fig. 2).

Discussion

Percutaneous penetration of pharmaceutical compounds is a complex biological process. Multiple factors govern the rate of percutaneous diffusion into body fluids (Scheuplein, 1980). Passive diffusion of drugs correlates well with various solubility factors reflected by the partition coefficients (Menczel & Touitou, 1989). Addition of various agents promotes percutaneous absorption of chemicals. As the galenic preparation (gel or cream; selection of transport enhancers) has a pronounced effect on transdermal absorption and penetration kinetics (Ziegenmeyer, 1982), it can be postulated that galenic differences also influence interindividual transmission potency.

In several cases adverse effects of androgen transfer after topical androgen application have been reported in infants and females. Yu *et al*. (1999) reported that over a period of months a 2-year-old boy developed virilization, including penile enlargement and growth of pubic hair and facial acne. This sexual development was induced by incidental and unintentional dermal exposure to a (not FDA approved, illegal) testosterone cream of which further ingredients are unknown. The cream was applied to his father's arm and back and probably also to sports equipment as part of a body-building regimen. Except for penile size, the other signs of virilization diminished several months after exposure was discontinued.

Androgenization of female partners of volunteers applying a testosterone gel preparation during contraceptive studies has been reported (Delanoe *et al*., 1984). Eleven of 17 female partners had elevated serum testosterone levels, in four cases hirsutism was observed. In one case the gel was applied by the female to her partner, in the other cases, contamination during intercourse or by shared clothes was considered most likely. In another case a women complained about hirsutism which was completely reversible when her male partner stopped using a testosterone cream for treatment of hypogonadism (Moore *et al*., 1988).

In study I we demonstrated that even after 8 h, a considerable amount of testosterone (approximately 50% of the applied dose) can be recovered from unwashed skin. In addition, a relatively high amount of testosterone can be transferred after skin contact. Washing of the skin considerably reduced the amount of testosterone which could be recovered, as after 30 min less than 15% of the original amount previously applied was recovered. These data *per se* indicate that a considerable amount of testosterone remains on the skin for a long period (> 8 h) and therefore can potentially be transferred to another person. However, as no further decline in the amount of testosterone recoverable from the skin was found after 10 min, when the alcohol had evaporated completely, it can be postulated that from this time-point onwards no further testosterone had penetrated the skin. Alcohol or comparable enhancer substances are essential for the penetration of testosterone; thus testosterone without alcohol, at least in the gel formulation investigated, seems to be unable to penetrate skin. Thus it is unlikely that after alcohol evaporation clinically significant amounts of testosterone can be transferred to a second person, female partner or child, resulting in unwanted adverse virilization.

In study II we investigated whether testosterone can be transferred to another person by extended close skin contact. Because of the high and fluctuating testosterone serum levels in healthy normal men, it is impossible to detect the relatively low increase in testosterone which could be estimated to occur in the worst case after close skin contact. For ethical and forensic reasons we did not want to expose females to testosterone because, although unlikely, virilization of the female volunteers could not be excluded. Hence the study was performed in healthy male volunteers in whom endogenous testosterone production had been suppressed by norethisterone enanthate, resulting in low and steady testosterone serum levels. A 24-h profile showed that only marginal variation in testosterone serum levels was observed over 24 h (mean testosterone serum concentrations ± SD, 2·4 ± 0·38 nmol/l). In study II we demonstrated that even without washing the skin, no increase in testosterone serum levels in the group of recipients was found, indicating that, after evaporation of the alcohol, no clinically relevant amount of testosterone can be transferred. Adverse effects of virilization of female partners and infants, reported after application of other transdermal androgen preparations, seem to be very unlikely in this preparation. Furthermore, as no additional testosterone is able to penetrate the skin after evaporation of the alcohol, patients can easily be told to wash the skin areas after the gel has dried, thus significantly reducing the amount of testosterone on the skin which could be transferred unintentionally. Washing the skin 10 min after gel application does not result in reduced testosterone serum levels being achieved after topical gel application (publication in preparation) and can be considered safe.

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