Biphasic Testosterone Delivery Profile Observed with Two Different Transdermal Formulations

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Purpose. Our long-term goal is to develop formulations for pulsatile testosterone (T) delivery. T has been reported earlier to show biphasic pharmacokinetics in humans by Mazer *et al*, as well as biphasic permeation across excised rat skin by our group. We examined two kinds of formulations to evaluate their delivery profiles and to assess whether differences in the formulation approach affect pharmacokinetics in animal models.

Methods. One formulation consisted of T and a polymer blend dissolved in isopropanol; administered by dispensing the solution on the skin to cast a film *in situ*. The other was an adhesive-dispersion patch. *In vitro* release from the patch was evaluated using a flow-through cell interfaced with an HPLC pump and UV detector. Single dose pharmacokinetics were evaluated in castrated Wistar rats and bonnet monkeys immunized against gonadotropin-releasing hormone to deplete endogenous T.

Results. Two maximas were observed in the T release profile from the patch and in serum concentration *versus* time profiles in both animal models on application of either formulation. The relative magnitudes of the two maximas and the time interval separating them were different in the case of each formulation.

Conclusions. Both formulations result in biphasic pharmacokinetics of T in the animal models studied. Discrete maximas presumably correlate with "burst" and "sustained" phases of drug release.

KEY WORDS: transdermal; testosterone; androgen supplementation; male contraception; pulsatile release.

INTRODUCTION

Transdermal testosterone (T) delivery for rational supplementation of androgen has been well received (1–3). Another possible application of T is in the development of a male contraceptive, where feedback inhibition of the hypothalamus-pituitary-gonadal axis by T is envisaged to render the subject infertile (4–6). The serum concentration profile of T in normal males incorporates a series of pulses within the 24 hour period (7). It is believed that the amplitude and frequency of these pulses are important in the feedback regulation of the release of gonadotropins (8,9).

A delivery system that seeks to mimic the physiological profile of T ideally needs to accomplish pulsatile delivery of the hormone. A paradigm for male contraception that seeks to disrupt endogenous hormone pulsatility by superimposing a pulsatile pattern of T delivery has also been proposed (10). Pulsatile delivery, however, is a difficult task for a system that relies on merely Fickian diffusion for controlling drug delivery.

We have reported the use of a liquid formulation for controlled transdermal delivery of testosterone earlier (11). This will be referred to as the *lotion* here. The other formulation used in these studies was an adhesive-dispersion patch described previously. A commercially available pressure-sensitive adhesive formulation was compounded with T and coated on to fabric (12). This report describes *in vitro* T release from the patch and single dose pharmacokinetics of both formulations in the castrated rat and a monkey model in which endogenous testosterone was depleted by immunological intervention.

MATERIALS AND METHODS

Testosterone BP was purchased from Fluka Laboratories, (Switzerland). All other polymers, chemicals and reagents were from Indian subsidiaries of Loba Chemie, Merck or Glaxo (Qualigens) and were of Laboratory Reagent grade. Emdilith DM-45 (Mafatlal Dyes and Chemicals, 193, Backbay Reclamation, Mumbai 400021, India) is a proprietary formulation based on a vinyl acetate terpolymer and used for preparing pressure-sensitive adhesive products. This was purchased from their New Delhi office (3/1, Hoechst House, Asaf Ali Road, New Delhi 110002, India) and used to prepare adhesive dispersion patches. Medication used in surgery and post operative procedure with animals was obtained from a retail Pharmacy and was for human use.

Formulations

A blend of water-soluble *poly*(vinyl pyrrolidone) (PVP) and *poly*(vinyl alcohol) (PVA) was standardized to yield a tacky film entrapping crystalline T when cast from an iso-propanol solution directly on to skin using a micropipette. Uniformity in the thickness and area of the film was achieved by the use of a simple applicator (a plastic bottle cap with a hole bored in its center) that restricted the height of the micropipette tip above the skin surface to a constant value. The thickness and area of the films thus cast were not estimated *in situ*, but a separate experiment in which films were cast on glass slides and evaluated gave an average ($\pm$S.D.) values of 0.036 ± 0.007 mm for thickness and 71.75 ± 4.35 mm^2 for area. Details of formulation, evaluation and *in vitro* skin permeation profiles studied using a flow-through cell have been reported earlier (11). The films cast from the lotion could not be tested for *in vitro* drug release, since they are composed of a water-soluble polymer blend.

For preparing the patches, T dissolved in *iso*-propanol was mixed with a standardized proportion of Emdilith DM-45 and the wet mass coated on a cotton fabric backing using an apparatus described earlier (12). This apparatus employs a synchronous motor to draw a web of fabric from underneath a coating knife. Material placed on the fabric before it passes under the knife is coated to a thickness determined by the clearance between the fabric and the knife edge. The coated tape is then dried at 45°C and a release liner applied to the adhesive surface. Patches used for *in vitro* drug release, rat and monkey pharmacokinetics reported here were either cut or punched out of a single strip of coated fabric. Their thickness was uniformly 0.439 $\pm$

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0.003 mm and areas were 156, 94 and 281 mm^2 respectively, corresponding to a mean (±S.D.) content of 5 ± 1, 3 ± 0.5 and 9 ± 1.5 mg. The composition of both formulations is shown in Table 1.

In Vitro T Release

Drug release from patches was studied *in vitro* using a flow-through diffusion cell interfaced to HPLC hardware (11). A Waters' 510 HPLC pump was interfaced to the cell and phosphate buffered saline (PBS, 50 mM, pH 7.4) containing 20% *poly*(ethylene glycol) 400 (PEG-400) was kept in a 1,000 ml conical flask placed in a 37°C water bath (Scientific Systems, New Delhi). Fresh PBS-PEG was drawn into the flow cell at 1 ml/min. Patches containing 5 ± 1 mg T were placed so that approximately 1 cm^2 of the drug-containing adhesive layer was in perpetual contact with buffer flowing through the cell. No barrier was present between the patch and the buffer. The outflow from the cell was routed to a 'discard' reservoir through a Waters' 490E UV detector set at 239 nm at a sensitivity of 3 AUFS and time constant of 1 second. The detector signal was recorded continuously by a Waters' 745B integrator.

A dummy or drug containing patch was then placed in the flow through cell and the response of the detector recorded over a period of 60 to 120 minutes. During this period, the integrator was programmed to print out values of the signal (recorder level) at 10 minute intervals. The printed values correspond to an "intermittent" sampling protocol, whereas the continuous trace represents "continuous" or real-time release. A representative example of such an experiment is reported here.

The Rat Model

Male outbred Wistar rats (n = 12) aged 6–8 weeks and weighing between 120–150 g were maintained by the Small Animal Facility of the National Institute of Immunology under WHO guidelines (equivalent to NIH Principles of Laboratory Animal Care) and were allowed pellet diet and water *ad libitum*.

Animals were castrated via an abdominal incision under ketamine anesthesia and allowed to recover for 15 days under analgesia for the first 3 days and topical antibiotic treatment for a week. On day 15 and 17, blood samples were withdrawn by retro-orbital puncture under ether anesthesia at 10 AM and were assayed to confirm the abolition of endogenous T. An area of the back was depilated using a hair remover lotion on day 17. An emollient cream was later applied to the depilated area.

Table 1. Composition of Lotion and Patch Formulations

Ingredients for lotion	Quantity	Ingredients for patch	Quantity
PVP 4,000	3.8 parts	Emdilith DM-45	8.7 parts
PVA 125,000	0.2 parts	Testosterone B.P.	3.1 parts
Light mineral oil	0.0125 parts	*iso*-propanol	*qs*[a]
Tween-20	0.002 parts		
Testosterone B.P.	4 parts		
iso-propanol	10 parts[a]		

[a] *Iso*-propanol added during compounding evaporates after application on skin in the case of the lotion and during coating and drying of coated tape in case of patches.

On day 21, 0.5 ml blood samples were withdrawn according to the following schedule: starting at 10 AM, samples were drawn at 15 minute intervals for the first two hours. Thereafter, the sampling times were at 4, 8, 12, 18 and 24 hours after application. Animals were under urethane anesthesia for the first 8 hours, and were bled under transient ether anesthesia at the next three time points. A dummy formulation containing all components except T was applied to the depilated area three days later and blood samples withdrawn as before. The site of application was liberally swabbed with *iso*-propanol after the experiment to remove any adhering formulation components. On day 28, 100 µl of the formulation containing 3 mg T was applied to the same area and samples withdrawn at aforementioned time points. The animals required further depilation before the patch could be tested. This was conducted on day 30. An identical sampling protocol was followed on day 35, after application of a patch containing 3 ± 0.5 mg T. A strip of surgical plaster covering the back and abdomen was required to secure the patch on the animals after they recovered consciousness. All 12 animals thus received each treatment: dummy, lotion and patch, separated by at least 7 days.

T was estimated in serum samples by radioimmunoassay (RIA) using reagents and protocol supplied by the WHO Matched Reagent Program (13). Fifty microliters of serum was assayed for the hormone and the lower limit of quantitation in this assay was 0.2 ng/ml. The intra-assay variation was of the order of 7% (data not shown).

The GnRH-Vaccinated Monkey Model

Four adult *Macaca radiata* (MRA) males of proven fertility, maintained by the Primate Research Center of the Institute under WHO guidelines, were recruited for the study after thorough evaluation of their pre-immunization T level at 10 AM and sperm counts in the mating season. These animals were immunized three times at monthly intervals with 200 µg doses of the gonadotropin-releasing hormone (GnRH)-diphtheria toxoid conjugate vaccine (14). This contraceptive/therapeutic vaccine neutralizes the GnRH produced by the hypothalamus. Such a blockade affects all further steps in the endocrine cascade, resulting in severe depletion of T.

Pharmacokinetics of T delivered by the formulations were studied in these animals upon confirmation of sufficiently high anti-GnRH antibody response, depleted serum T levels and severe oligospermia. Blood samples were obtained by venous puncture, without anesthesia, using a 23 GA hypodermic needle as per the following schedule. Starting at 10 AM, 1 ml samples were obtained at 0, 0.5, 2, 4, 8, and 12 hrs. Another sample was collected either at 22 or 24 hrs. In the first experiment, a dummy lotion containing no drug was applied on the volar surface of the forelimb of all animals. Five days later, a patch containing 9 ± 1.5 mg T was applied at 10 AM to a similar site and secured with several rolls of surgical plaster. Blood samples drawn as before. After another 5-day wash-out period the lotion was evaluated by applying 3 × 100 µl doses of the lotion (containing 9 mg T) together at 10 AM, and sampling as described.

Data Analysis

The extent of T delivered was assessed by comparing the areas under the curve (AUC) obtained by integrating the curves

using Simpson's rule. The presence of multiple peaks was investigated by means of iteratively fitting a multiple Lorentzian curve to the concentration-time profile using the program MicroCal Origin, version 2.0. Such a function has the general form:

$$y = 2A(w/p_i)(w2 + 4(x - x_0)^2)$$

Where y is the concentration at any time, x and x_0 are the values of time at the center of the putative peak at that time and time 0 respectively, A is the area under the putative peak, w is the width of the peak at half-height and p_i is the initial value of the fitting parameter. The nature of the profile derived from this function and the goodness of fit to the experimental data can indicate the validity of the assumption that a peak does lie at the point on the profile where it is indicated. The aim of the curve-fitting exercise was to apply an objective method of peak detection. Merriam (15) has cautioned against accepting apparent peaks as valid without independent evaluation.

RESULTS AND DISCUSSION

Representative results of *in vitro* T release from a patch are shown in Figure 1. Patches containing no T did not cause any deviation from the baseline apart from a drift. This figure serves to highlight the difference in the profiles obtained by "intermittent" sampling as represented by the values of the recorder level printed out and "continuous" monitoring as reflected in the trace of the recorder. The biphasic nature of the delivery profile was more efficiently highlighted by the trace of the recorder. The continuous trace is an efficient and time-saving method of studying the release profile, and can offer an advantage when seeking to track the appearance and resolution of transient behavior. For instance, it would be difficult to obtain closely spaced samples in the region between 0 and 5 minutes that would reflect the appearance and decay of burst release. Varying parameters such as the flow rate or buffer composition could, of course, retard the burst, but would necessitate the expenditure of more time in conducting the experiment.

Prior to the commencement of the *in vivo* studies, endogenous T in the rat model was observed to have been depleted to very low or undetectable levels by day 15 after castration. No T could be detected in samples drawn on Day 21 after castration (data not shown). This confirmed that any changes consequent to application of test formulations could be ascribed to the administered T rather than endogenous hormone. Application of a dummy lotion or patch to the animals did not result in any quantifiable increase in serum T levels. The serum T concentration *versus* time profile following the administration of a single dose of 3 ± 0.5 mg is shown in Figure 2 along with the profile derived from a multiple Lorentzian peak fitting

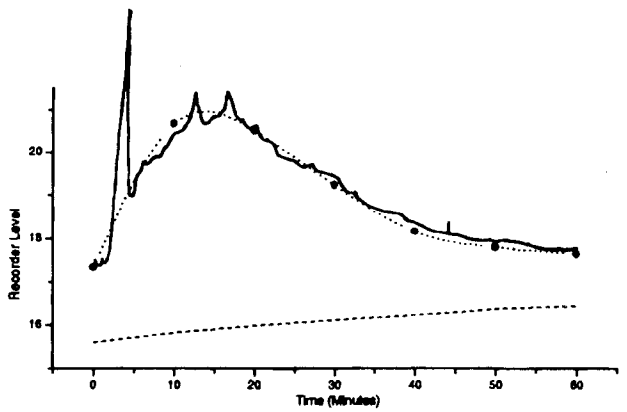

Fig. 1. Real-time trace of T release from a patch (solid line) as recorded by the HPLC integrator shows a marked difference in the relative amplitudes of the peaks corresponding to initial and delayed drug release as compared to the curve (dotted line) plotted using recorder level values returned every 10 minutes to represent intermittent sampling in the same experiment. A dummy patch tested similarly showed a drifting baseline (dashed line).

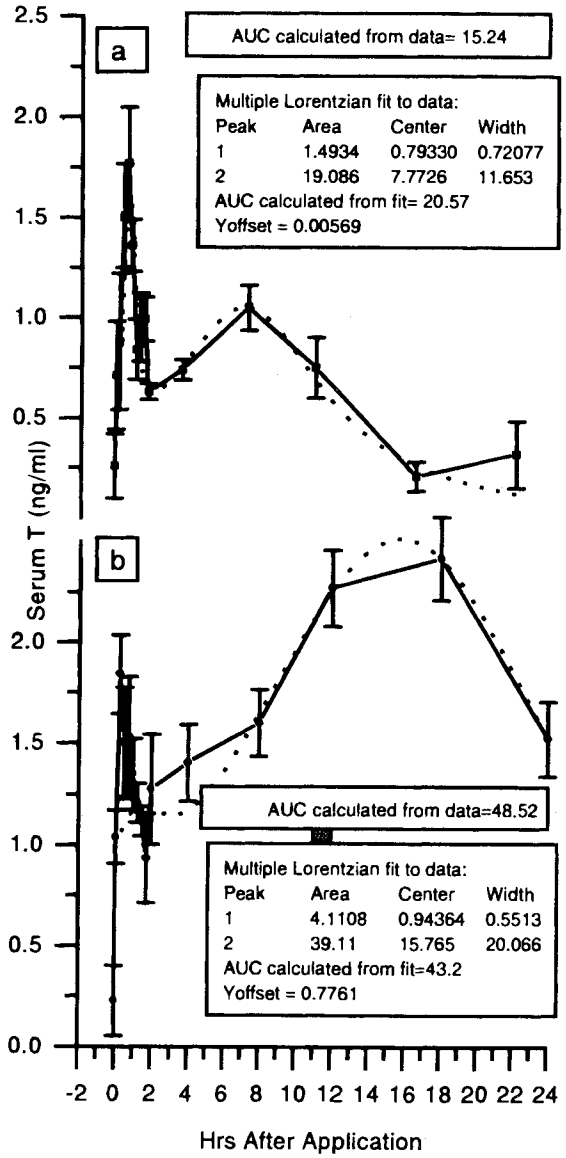

Fig. 2. Serum T profile after application of (a) the lotion and (b) the patch formulations containing 3 mg T. *Solid line*: point-to-point profile using mean values (±SD, n = 12). *Broken line*: multiple Lorentzian function fit to mean values closely approximates the solid line. *Inset*: fitting parameters indicating properties of peaks detected and AUC calculated.

function. The fitting exercise demonstrated that an objective assessment of the profile by a numerical analysis method substantiates the subjective observation of 2 maximas in the profile by the human eye. Despite some discrepancies in the AUC and t_{max} values calculated from the raw data and the fit function, both data sets generally agree (see inset for values). Reported values of T serum concentrations are within 12% coefficient of variation between replicates.

Figure 3(a) depicts the results of application of a dummy to the GnRH vaccinated monkeys. The depletion in T concentration and abrogation of pulsatility is clearly visible in all animals, despite the presence of an artifactual peak in the first few samples drawn from MRA 218 and 392. There is no obvious reason why T should be elevated in these samples. Single-point samples drawn to confirm the abrogation of T levels in these animals during the maturation of the immune response to the vaccine were taken around this time of the day. These samples showed highly depleted T in comparison to pre-immunization levels, strongly correlating with the increasing anti-GnRH antibody titer (data not shown). No procedural fault is evident at the level of the RIA, since all internal controls (ether blanks and quality-control tubes containing human serum of known T concentrations) were consistent. For the purpose of interpreting the results reported here, it is obvious that these "artifactual" concentrations are still much below those observed on application of the formulations as shown in Figure 3(b) and 3(c). These panels illustrate the effect of administering formulations containing 9 ± 1.5 mg of T to the same animals. Not only is the rise in serum concentration apparent, but the unusual feature of the profile dipping and rising is clearly observed in each case except MRA 186 (Fig. 3b). This animal did not exhibit the later maximum and thus showed a significantly lower AUC.

Biphasic diffusion behavior of T has earlier been reported by Mazer *et al.* (1). This group commented on the splitting of the diffusion pattern of T into a rapid phase lasting about 8 hours, followed by a phase of slower release over the next 12 hours. At least one other report (16) describes biphasic pharmacokinetics of another steroid—estradiol, from a transdermal patch in human patients. The two peaks are observed 36 and 84 hours after application respectively. Although an analysis of the formulation factors contributing to the above observations is not within the scope of this article, it is encouraging to note that the reported phenomenon is evidently observed elsewhere as well.

Earlier studies and specially those of Schaison *et al.* (17) employing androgens in a hydro-alcoholic gel substantiate the rapid bioavailability of topically applied steroids. These studies employ a design in which patients are instructed to take a shower 10 minutes after applying the formulation. Satisfactory serum DHT concentrations are achieved in this relatively short time of exposure.

At this time we can only speculate as to the reason why the nature of the bioavailability profile from the reported formulations is biphasic. In terms of Fickian diffusion, at least two distinct diffusivities are displayed by T in these formulations. This can occur if the T incorporated in these systems separates into rapidly- and slowly-diffusing domains. The recrystallization of T from a highly supersaturated solution in presence of nucleation-inhibiting polymeric chains seems a likely mechanism to explain how such a separation of domains could occur. The rapid evaporation of the solvent causes high rates of supersaturation to develop. Imperfect, dendritic crystals are invariably formed under such conditions (18). PVP has long been known to have an "anti-crystallizing" effect on various compounds. Polymer chains interact with the soluble compound and hinder the solute-solute interactions that go into making up the crystals (19,20). A crystallizing bed that is exposed to rapid supersaturation in the presence of polymer chains would certainly contain precipitates that do not exhibit the 3-dimensional structure expected of well-formed crystals of the solute.

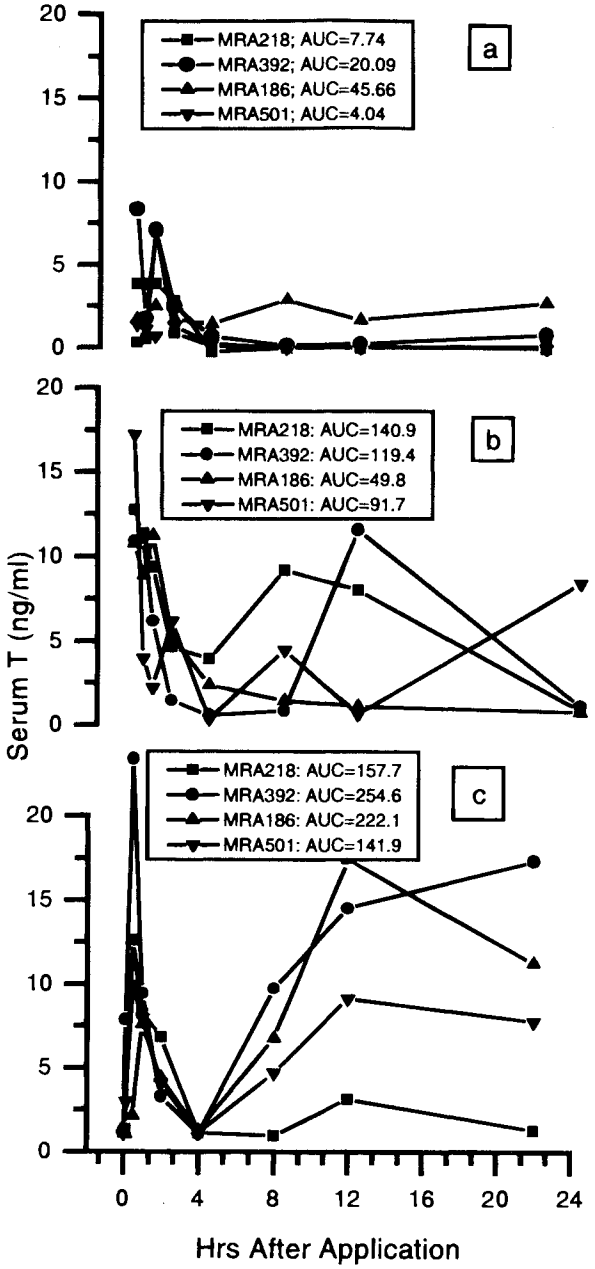

Fig. 3. Serum T concentration over the sampling period in the GnRH-vaccinated bonnet monkeys (MRA). MRA 218, 392, 186 and 501 refer to individual animals. Bioavailability is indicated in terms of the area under the concentration-time curve (AUC). Background serum T after application of dummy lotion (a) was much lower than serum T profiles after application of lotion containing 9 mg T (b) or patch containing 9 ± 1.5 mg (c).

We are currently trying to examine this possibility using X-ray diffractometry and electron microscopy. Kurnik and Potts have modeled the effect of crystal size on dissolution and diffusion behaviour (21). Their work indicates that smaller crystal size can result in significant increases in the release rate, although they have not observed this to impact on the percutaneous permeation of estradiol, wherein rate control is primarily exercised by the skin.

Our view on the observation of biphasic pharmacokinetics can now be stated as follows. Since free T has a short half life in circulation, rapid bioconversion of the exogenously supplied hormone enables the changes in T serum concentration to be observed. The large amount of T diffusing into the skin as a burst within a few minutes after application of the formulations reported here would establish a "reservoir" of T in the lipophilic environment of the stratum corneum. This would lead to sustained diffusion of the drug by first order kinetics, reflected in declining serum concentration as time progressed. However, Figures 2 and 3 show a significant rise a few hours after application of drug containing formulations. The rise can be accounted for by the contribution of matrix controlled release of T from the polymer matrices in addition to release from the skin reservoir. *In vitro* skin permeation studies with the lotion formulations bear out this conclusion since pure drug in solution yields a diffusion profile markedly different from situations in which polymer blends are also incorporated in the solution (11). The initial peak can be accounted for by the phenomenon of burst release. The later peak, however, can only result from a subsequent phase of delivery, incorporating a lag rather than a burst.

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