J. Pharm. Pharmacol. 1987, 39: 90–95
Received July 30, 1986

Influence of solvent on the availability of testosterone propionate from oily, intramuscular injections in the rat

M. K. AL-HINDAWI, K. C. JAMES*, P. J. NICHOLLS

Welsh School of Pharmacy, University of Wales Institute of Science and Technology, P.O. Box 13, Cardiff CF1 3XF, UK

The suggestion that the biological response to an oily intramuscular injection of testosterone ester is regulated by rapid accumulation of the steroid in body fat, followed by a slow release, has been tested by comparing the release rates of ^{14}C-labelled testosterone propionate from different solvents following intramuscular injection into rats. Disappearance from the injection site was rectilinearly related to in-vitro partition coefficients, but elimination of radioactivity in urine and faeces was significantly longer, and the same for all four solvents. Testosterone and testosterone propionate were found in equal concentration in body fat, 2 and 3 days after injection, but their concentrations were too low to form an effective depot. It is suggested that the delay in release, and the independence of the delay on the nature of the solvent is a consequence of biliary recycling of testosterone.

When testosterone is used therapeutically, it is normally administered intramuscularly as an ester in oily solution. It is given in this way because the free alcohol exerts only a small, transient biological effect, while the esters evoke effective, prolonged responses, the duration of action increasing with increasing lipophilicity of the ester (Miescher et al 1936). The rate-controlling process has been considered to be transfer of the ester from the globules of the injected dose in the muscle to the plasma, by a partitioning process. However, there is evidence (James et al 1969) that the hormone is released from the muscle too rapidly to account for the slow rate of elimination from the whole animal. It has been suggested (Armstrong & James 1980) that testosterone and/or its ester released into the plasma, is taken up and stored in another depot, probably body fat, from which it is slowly discharged. Thus the rate of transfer from a globule of the injected lipid dose form in the muscle should vary with the nature of the solvent, and be related to the partition coefficient of the solute between the solvent and plasma, while release from a second depot should be independent of the solvent. In the present study, intramuscular injections of testosterone propionate in four different solvents have been administered to rats, and biological responses compared, in an effort to identify the mechanism(s) upon which bioavailability depends.

* Correspondence.

MATERIALS AND METHODS

Solvents

Ethyl oleate BP (BDH Ltd), 'Specially Purified' n-octanol (BDH Ltd), isopropyl myristate BP (Fluka AG) and light liquid paraffin (Macarthys Ltd) were used, without further purification, as injection vehicles.

Preparation of [4-^{14}C]testosterone propionate

Unlabelled testosterone (1 mg) was added to 9·25 MBq of [4-^{14}C]testosterone in benzene, purchased from the Radiochemical Centre, Amersham. The solvent was evaporated under nitrogen at 50 °C. Propionic anhydride (0·2 mL) and 1 mL pyridine were added, and the mixture heated over a water bath for 4 h. About 5 g of ice was added, and the precipitate which formed removed by filtration, and washed with water until the filtrate gave no colour change with methyl red. Benzene (2·2 mL) was added to the final product, so that each 1 mL of solution contained 13·6 mg of testosterone propionate with 3·7 MBq of activity and evaporated off before the injections were prepared. Purity was checked by TLC, followed by scanning with a Tracerlab 4π radiochromatogram scanner.

Preparation of injections

Unlabelled testosterone propionate (17 mg) was weighed into an ampoule containing labelled material, when required, and 0·2 mL of the injection

solvent added. The filled ampoule was reweighed and sealed.

Sample preparations for radioactivity determinations
Methanolic potassium hydroxide solution (2 M, 7 mL) was added to each complete rat gastrocnemius muscle and heated at 80 °C for about 7 h, until the tissue was completely digested. The digest was adjusted to 10 mL with methanol, and 1 mL of the dilution added to 10 mL of scintillator. Urine (1 mL) was added directly to 10 mL of scintillation fluid. Faeces were homogenized in 70 mL of 2 M methanolic potassium hydroxide, and then maintained at 60 °C for 24 h. The liquid digest was decanted off and fitered through Celite 80–120 mesh. The filtrate was reduced to about 5 mL, accurately measured in a rotary evaporator, and 0·1 mL aliquots added to 10 mL of scintillator.

Blood was collected by cardiac puncture at predetermined intervals from rats in groups of 2 or 3, under ether anaesthesia. Samples were immediately transferred to heparinized tubes, and centrifuged at 3000 rev min^{-1}. Samples (0·2 mL) of plasma were pipetted into scintillation vials containing 15 mL scintillation cocktail. The accuracy of each method was checked with spiked samples. Radioactivity was calculated on the basis that total plasma is 4% of body weight (Archer 1965).

For experiments in which radioactivity in fat was determined, doses of 0·1 mL containing 1 mg of testosterone propionate with 370 kBq of radioactivity, dissolved in isopropyl myristate, were injected into the right gastrocnemius muscles of a group of rats. Two or three rats were killed 2 and 3 days after injection, and a sample of perirenal fat was removed. The radioactivity of each sample was determined by weighing 90 to 150 mg into a scintillation vial. NCS tissue solubilizer (2 mL) (Radiochemical Centre, Amersham) was added and the vial maintained at 55 °C for about 72 h, until the specimen was fully digested. After cooling, the contents were acidified with a few drops of glacial acetic acid, and 10 mL of scintillation fluid added. Control samples were treated in the same manner. Since it was impossible to remove the total weight of body fat, the fraction of the tissue in normal adult human (16% b.wt) given by Diem & Lentner (1971) was used to estimate the carbon-14 activity in total body fat.

Other samples of fat were extracted in an ultrasonic bath for three 16 h periods with a total of 10 mL acetone. Each time, the extract was removed and replaced with fresh acetone. Unlabelled testosterone and testosterone propionate were added to the combined extracts after the bulk of the acetone had been removed by evaporation. This solution was applied as a continuous band to silica gel plates, with fluorescence at 254 nm. Plates were developed three times by the ascending method, with 25% ethyl acetate in light petroleum (b.p. 60–80 °C). The plates were taken out and dried after each development, then replaced in the tank. Two strong and three weak bands were observed under UV light, and the two strong bands identified with testosterone and testosterone propionate. The silica was scraped from each position, and added to 600 mg of silica (Cab-o-sil M-S) in a scintillation vial. Scintillator (12 mL) was added, and the vial shaken vigorously, left for 24 h for stabilization, and then counted.

Determination of partition coefficients
Carrier-free [^{14}C]testosterone propionate in ethyl oleate (5 mL) was pipetted into 10 mL of water in a 50 mL jacketed tap funnel. The water had been previously equilibrated with ethyl oleate, and heated to 37·8 °C. Water at 37·8 °C was circulated through the jacket of the funnel. The mixture was shaken vigorously for 8 h, then left to stand for 24 h. Samples (0·1 mL) of the ethyl oleate phase, and the aqueous phase were withdrawn and added to 3 mL of scintillator. Partition coefficients were calculated as the ratio of the radioactivities. The procedure was repeated with isopropyl myristate, light liquid paraffin and octanol. Three to five determinations were carried out for each solvent.

Half-life determination
In muscle. A 0·1 mL dose of labelled testosterone propionate solution was injected into the right gastrocnemius muscles of a group of male Wistar rats. After predetermined intervals, groups of three rats were killed, and their left and right gastrocnemius muscles removed. The left gastrocnemius muscles of three rats, removed at zero time, were used as blanks.

In whole rats. Groups of three rats were injected in the manner described above, and kept separately in metabolism cages (Jacon's metabowl). Urine and faeces were collected daily for 17 days. Rats injected with pure solvent were used as controls.

Determination of biological response
Male Wistar rats (40 to 60 g) were castrated under ether anaesthesia. On the 14th day after castration, each animal was injected with testosterone propionate in 0·1 mL of solution. Testosterone propionate

(0·75 mg) was injected in ethyl oleate, octanol, and isopropyl myristate, but only 0·5 mg was administered in light liquid paraffin because of the limited solubility in this solvent. Four 0·025 mL doses, two in each leg, had to be given with the octanol solution, but with the other solvents, one dose was administered. For each solvent, groups of three rats were decapitated after predetermined intervals, and prostate glands and seminal vesicles removed and weighed. Mean weights were plotted against time, and the areas under the plots, less control area, were measured and used to assess androgenic activity (Chaudry & James 1974).

Liquid scintillation counting

This was performed using an LKB 1215 Rackbeta II scintillation spectrometer. Quench correction was by the channels ratio method. For solutions in organic solvents the cocktail of Hall & Cocking (1965) was used and for aqueous solutions that of Graham & Nicholls (1959) was used.

Histology

Octanol (0·1 mL) was injected into the left gastrocnemius muscle of a male Wistar rat. After 1 day, the animal was killed and the injected tissue dissected out, together with the corresponding region of the left, untreated muscle. The procedure was repeated on two other rats, one with 0·1 mL of ethyl oleate and the other with 0·025 mL octanol. The six tissues were examined histologically.

RESULTS AND DISCUSSION

Partition coefficients of testosterone propionate between water and the four solvents used in the biological experiments are given in Table 1. Determinations were carried out with carrier-free solutions, to ensure that thermodynamic coefficients associated with infinitely dilute solutions, were obtained. A wider range of partition coefficients would have been preferable, but the number of suitable solvents which are immiscible with water and also non-irritant, is extremely limited. Even among the four solvents used, octanol was not entirely satisfactory, because injection volumes approaching 0·1 mL showed some evidence of oedema. It was for this reason that four 0·025 mL doses were administered.

Histological examination of the muscle treated with 0·1 mL octanol revealed necrosis of myocytes with an acute inflammatory cell exudate and oedema, accounting for the observed swelling. In contrast, the muscles treated with ethyl oleate and 0·025 mL octanol showed no damage in comparison with the controls.

Half-lives for elimination from muscle are given in Table 1. The first three were obtained from plots of log mean activity remaining in the muscle against time on 0, 1, 2 and 3 days. Good rectilinear correlations were obtained; Fig. 1 is an example. The half-life for the ester in ethyl oleate was less than that observed previously (James et al 1969). This was attributed to the different intramuscular site used in the present work. With light liquid paraffin, the rate of elimination was so rapid that muscles had to be removed after 0, 3, 6 and 9 h.

In preliminary calculations of release from whole rat, total radioactivity in urine and faeces was plotted against time, and found to reach a limiting cumulative activity in about 5 days. These values were taken

Table 1. Partition coefficients at 37 °C and elimination half-lives of testosterone propionate. Each value is the mean of 3 results; standard deviations are given in parentheses. No standard deviation is quoted for the whole body result with light paraffin, because it is the mean of 2 determinations. The faeces sample from the third rat was lost on day 2, but the result for urine levels alone (17·2 h) indicates that a similar result could be anticipated for this animal.

Solvent	Partition coefficient K_p ($\times 10^{-3}$)	Half-life (h) Muscle	Whole body
Ethyl oleate	6·3 (0·4)	10·3 (0·8)	19·1 (2·0)
Octanol	5·3 (0·5)	9·7 (0·1)	19·3 (1·6)
Isopropyl myristate	4·3 (0·2)	7·8 (0·3)	22·1 (3·1)
Light liquid paraffin	1·5 (0·3)	3·2 (0·3)	18·1

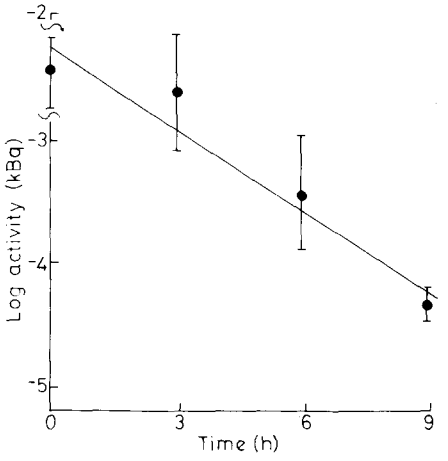

FIG. 1. Residual radioactivity in muscle following injection of [^{14}C]testosterone propionate in 0·01 mL of light liquid paraffin.

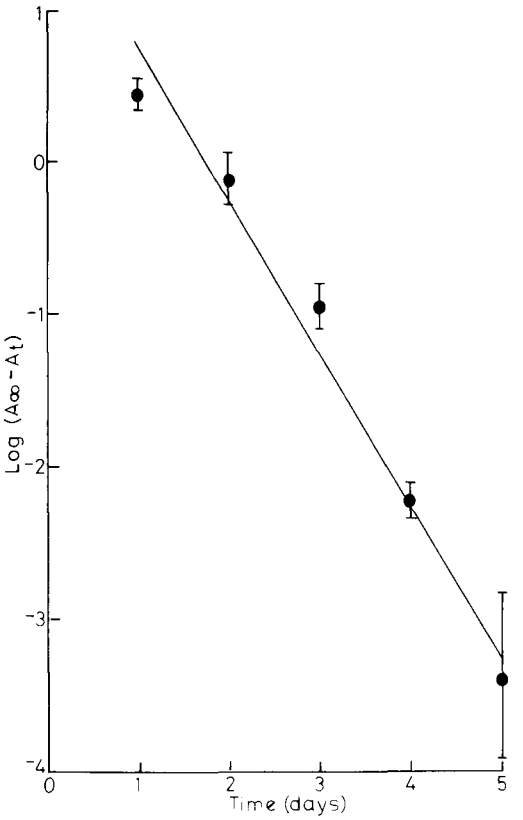

FIG. 2. Residual radioactivity in whole rat, determined from combined urine and faeces levels, following injection of [^{14}C]testosterone propionate in 0·1 mL of light liquid paraffin.

as radioactivities at infinite time (A_∞), and ln ($A_\infty - A_t$), where A_t is cumulative activity at time t, was plotted against time. Good rectilinear plots were obtained; Fig. 2 is an example. The mean half-lives obtained from these plots are given in Table 1. Correlation of elimination rate constant ($0{\cdot}693/t_{\frac{1}{2}}$) in muscle with partition coefficient was rectilinear and yielded equation 1,

$$-\ln k = 0{\cdot}831 \ln K_p - 4{\cdot}532 \quad \overset{n}{4} \quad \overset{r}{0{\cdot}998} \qquad (1)$$

in which k is the first order rate constant, and K_p the partition coefficient, and suggests that the rate of elimination from muscle is dependent on a partitioning process between the globules of injection in the muscle and the plasma. In contrast, the elimination rate from whole rat is independent of the solvent, suggesting that the duration of biological action is not controlled by the release of the drug from the injection, which forms a depot in the muscle.

The overall androgenic activities, expressed as the area under the plots of organ weight against time, are shown in Table 2. Time of maximum effect is the day upon which the organ weight was greatest. The results given in Table 2 support the conclusion derived from Table 1, in that the results for ethyl oleate, octanol and isopropyl myristate are indistinguishable, despite a moderate range of partition coefficients. Light liquid paraffin appears to be an exception and will be discussed later. This solvent gave shorter times of maximum effect and the overall androgenic activities were also less than the others, even when the smaller dose is taken into consideration.

There is evidence (Armstrong & James 1980) suggesting that body fat can serve as a depot for lipophilic drugs. With this in mind, samples of perirenal fat were examined 2 and 3 days after injection of labelled testosterone propionate in ethyl oleate. Five samples were taken, and gave a mean radioactivity corresponding to only 0·3% of the injected dose in the entire body fat. It thus follows that a very low proportion of radioactivity is deposited in body fat. The identity of the radioactivity was investigated by TLC, results of which are given in Table 3, and show that on day 2 and day 3 after injection, testosterone and testosterone propionate were present in fat in equal quantities. The remaining activity probably represents metabolites, but these were not identified.

Samuels (1966) pointed out that a partition coefficient favouring lipids leads to concentration in adipose tissues, because the partition coefficient is directly proportional to the rate of entry into body fat and inversely proportional to the rate of release. Partition coefficients between ethyl oleate and water (Roberts 1969) suggest that testosterone propionate would be taken up by fat 10 times more readily than testosterone. Hence, to attain an equal level in the fatty tissues, testosterone must be present in the plasma at 100 times the concentration of the propionate and on its release from the muscle, only 1% will remain unhydrolysed and pass into the fat un-

Table 2. Overall androgenic activities (OAA) and times of maximum effect (TM).

	Prostate		Seminal vesicles	
Solvent	OAA (mg days)	TM (days)	OAA (mg days)	TM (days)
Ethyl oleate	54·1 (20·4)	3–4*	83·7 (24·0)	4
Octanol	48·0 (11·2)	4	74·7 (14·1)	4
Isopropyl myristate	55·1 (10·7)	4	102·0 (15·6)	4
Light liquid paraffin	17·2 (6·8)	2	36·9 (9·3)	2

* Identical results on days 3 and 4.
The figures in parentheses are 95% confidence limits.

Table 3. Distribution of radioactivity in body fat.

R_F value	0	0·077	0·194*	0·356	0·484	0·633†	1
Day	Disintegrations per minute						
2	198·7	63·9	477·8	65·3	44·5	472·0	0·0
3	73·6	–	181·7	95·3	38·9	276·1	12·5

* Testosterone band.
† Testosterone propionate band.

Table 4. Plasma concentrations.

Time after injection (h)	3	6	12
Solvent	% of total initial radioactivity per mL of plasma		
Ethyl oleate	0·50	0·42	0·43
Light liquid paraffin	0·85	0·97	0·39

changed. This is in agreement with observations on other esters (Jorgensen et al 1971; Aaes-Jorgensen et al 1977).

The difference between the half-lives in whole body and muscle could be due to entero-hepatic circulation, whereby the free alcohol passes into the intestine with the bile, and is absorbed into the circulation for further uptake by the liver, metabolism and excretion. If this is the delaying step, similar whole body half-lives and times of maximum effect, independent of the solvent used, would be anticipated. Entero-hepatic circulation as a possible reason for the protracted action of steroid esters has also been suggested by Masuoka et al (1979), and for other drugs by Thomas (1981). James et al (1969) determined the biological half-lives of testosterone and some of its esters in muscle and whole body, following i.m. injection into rat. Examination of their results reveals that the difference between the half-lives for testosterone is 1·96 days, which is similar to that for the rest of the homologous series studied, with the exception of the valerate, for which the effect was longer. The constant delay for testosterone and its lower esters implies that it is due to the hepatic circulation of free testosterone, and that the half-life in whole rat is a composite of a rate-determining step involving release from the muscle plus a constant delay involving biliary recycling.

Only one ester, the propionate, was investigated in the present study. Esters with slower hydrolysis rates and higher lipid solubilities could find their way to the body fat in higher concentrations. This could be the reason for the different behaviour of the valerate observed in previous results (James et al 1969). Significant quantities of steroid have in fact been found in body fat after i.m. injection (Plotz & Davies 1957; Steinetz et al 1967).

A possible reason for the anomalous behaviour of the testosterone propionate in light liquid paraffin is suggested by Table 4, which shows the plasma concentrations 3, 6 and 12 h after administration of injections in ethyl oleate and liquid paraffin. The plasma concentrations following injection of the light liquid paraffin solution are initially about double those obtained with ethyl oleate, providing greater scope for elimination by liver and kidney. This is confirmed by the marked drop in plasma concentration 12 h after injection of the light liquid paraffin solution, and also by the observation that 49% of the radioactivity was excreted in urine and faeces during the first day following injection, compared with 15% for ethyl oleate. Isopropyl myristate gave similar results to ethyl oleate. The half-lives in whole rat given in Table 1 were obtained from the cumulative increase in radioactivity in urine and faeces following these initial results, so that although the initial results for light liquid paraffin did not affect the slope, and hence the half-life, the initial radioactivity from which the plot of $\ln(A_\infty - A_t)$ against time began, was lower. The high elimination during the first day, could also account for the low androgenic response and shorter time of maximum effect obtained with the light liquid paraffin.

Acknowledgements

We are grateful to Dr Mary Tucker of Imperial Chemical Industries plc, Pharmaceuticals Division, for carrying out the histological examination.

REFERENCES

Aaes-Jorgensen, T., Overa, K. F., Bogeso, K. P., Jorgensen, A. (1977) Acta Pharmacol. Toxicol. 41: 103–120

Archer, R. K. (1965) Haematological Techniques for use in Animals, Blackwell Scientific Publications, Oxford, p. 120

Armstrong, N. A., James, K. C. (1980) Int. J. Pharm. 6: 185–193

Chaudry, M. A. Q., James , K. C. (1974) J. Med. Chem. 17: 157–161

Diem, K., Lentner, C. (1971) Scientific Tables, 7th ed, Ciba-Geigy Ltd, Basle, p. 517

Graham, J. D. P., Nicholls, P. J. (1959) Br. J. Pharmacol. Chemother. 14: 35–39

Hall, T. C., Cocking, E. C. (1965) Biochem. J. 96: 626–633

James, K. C., Nicholls, P. J., Roberts, M. (1969) J. Pharm. Pharmacol. 21: 24–27

Jorgensen, A., Overa, K. F., Hansen, V. (1971) Acta Pharmacol. Toxicol. 29: 339–358

Masuoka, M., Shikata, M., Fuziwara, R., Nakayama, R. (1979) Acta Endocrinol. 92 (suppl. 229): 24–35

Miescher, K., Wettsein, A., Tschopp, E. (1936) Biochem. J. 30: 1977–1989

Plotz, E. J., Davies, M. E. (1957) Proc. Soc. Exp. Biol. Med. 95: 92–96

Roberts, M. (1969) Ph.D. Thesis, University of Wales

Samuels, L. T. (1966) in: Pincus, G., Nakao, T., Tait, J. F. (eds) Steroid Dynamics, Academic Press, New York and London, pp 385–391

Steinetz, B. G., Meli, A., Giannina, T., Beach, V. L. (1967) Proc. Soc. Exp. Biol. Med. 124: 1283–1289

Thomas, G. H. (1981) in: Hathaway, D. E. (ed.) Foreign Compound Metabolism in Mammals, Vol. 6, Royal Society of Chemistry, London, pp 278–290