J. Pharm. Pharmacol. 1980, 32: 810–814 Received October 31, 1979

Availability of tritium from non-aqueous solutions of [1,2-^{3}H]methyltestosterone, administered orally to rats

K. C. JAMES*, P. J. NICHOLLS AND L. M. SANDERS†

Welsh School of Pharmacy, University of Wales Institute of Science and Technology, King Edward VII Avenue, Cardiff CF1 3NU, U.K.

The solvent–water distribution coefficients of 17α-methyltestosterone have been determined in selected alkanes, alkanols and alkane–alkanol blends. The oral availability of tritium from solutions of [^{3}H]methyltestosterone in these solvents and blends was studied in rats by measuring radioactivity in plasma, faeces and urine. Disappearance of radioactivity from the gut and biliary recirculation of ^{3}H were also examined. Variation of the solvent led to marked changes in the absorption of radioactivity, suggesting a possible method of varying bioavailability from soft gelatin capsules. Neither distribution coefficients nor tritium concentrations changed significantly from one homologue to another, but there was a marked difference when the alkane series was compared with the alkanol series. Blends of octane and octanol, together with the pure solvents, provided a suitable spread of distribution coefficients and tritium concentrations, but no simple relationship could be established between the two properties.

Traditionally, semi-synthetic steroids for oral use have been formulated in tablet form, but the problems associated with their manufacture are increasing as more potent steroids are discovered. It is difficult to mix tablet masses homogeneously when one component is in low concentration. Faint & King (1970) performed single tablet assays on 7 different oral contraceptive formulations, where the declared oestrogen content was 50 to 100 μg per tablet. In the best formulations, 5% of the tablets were more than 5% outside the declared composition, and in the worse 95% exceeded ±5% and 15% exceeded ±20%. The solution inside a soft gelatin capsule is molecularly dispersed, so that there are no mixing problems and the dose delivered is dependent entirely on the volume of solution in the capsule. The soft gelatin capsule therefore appears to be a more suitable presentation for steroid hormones than the tablet, provided that bioavailability from the capsule is adequate. Although this aspect has been established by Alibrandi et al (1960) and Bruni et al (1966, 1970, 1973), there have been no investigations of the influence of the solvent on the absorption of the drug.

The process of absorption, following oral administration of an encapsulated drug dissolved in a water-insoluble solvent, can be divided into three stages, the period leading to the rupture of the shell and release of the contents, passage of the drug from the solution into the gut contents, and absorption through the gut wall. The time involved in the first stage will be constant, provided that the composition and dimensions of the shell are the same for each, and that the solution does not react with the shell. Furthermore, assuming that the gut contents before administration of a capsule, are always the same, the only way in which the nature of the solvent can affect the third stage will be through its influence on the concentration of drug in the aqueous phase. The availability of tritium from [1,2-^{3}H]17α-methyltestosterone in rats was investigated with these points in mind. Solutions in a series of organic solvents were used without encapsulation, and bioavailability data compared with distribution coefficients of the steroid between the solvents and water.

* Correspondence.
† Present address: Syntex Research, Division of Syntex (USA) Inc., 3401 Hillview Avenue, Palo Alto, Calif, 94304, USA.

MATERIALS AND METHODS

Materials

Methyltestosterone. Labelled methyltestosterone was prepared at the Radiochemical Centre, Amersham, by partial tritiation of 17α-methyl-17β-hydroxyandrost-1,4-dien-3-one (methandienone) using the method of Quincey & Gray (1966). It had not been purified. Carrier methyltestosterone was a gift from Organon Laboratories Ltd. and was used without further purification.

Solvents. Purified solvents were obtained from BDH Ltd., and the Aldridge Chemical Co. and used without further purification. All had declared purities in excess of 98%.

Chromatography

Whatman No. 1 medium flow paper was used. The Bush (1961) reverse phase method was employed, using the descending technique. Precoated F_{254} silica gel plates, 200 × 200 × 0·25 mm (Macherey-Nagel & Co.), were used for t.l.c. Spots were located under u.v., and confirmed by spraying with 50% antimony trichloride in glacial acetic acid. Of 11 systems studied, light petroleum–ethyl acetate (50:50 v/v) on silica gel gave the best separation, and provided a medium from which the steroids could be eluted easily. This was adapted for purification of labelled methyltestosterone.

Purification of labelled methyltestosterone

5 mCi of crude [^{3}H]methyltestosterone in 2 ml benzene was applied in a line 2 cm from the edge of the t.l.c. plate which was run, and radioactive zones located by means of a spark chamber (Birchover Instruments Ltd.). Two clear bands were obtained, corresponding to the R_F values of methyltestosterone (0·32) and methylandrostanolone (0·49). Each zone was scraped into a Soxhlet thimble, and the plate re-examined in the spark chamber to ensure complete removal of the two bands of radioactivity. The thimbles were then extracted for 30 h with benzene.

Distribution coefficients

For each determination, 250 to 750 μl of tritium labelled methyltestosterone solution containing 10 μCi ml^{-1}, was added to 15 ml of water and 10 ml of organic solvent in a water-jacketed tap funnel. Funnels were maintained at 25 °C and shaken gently overnight. After standing for at least 24 h, to allow clarification of the aqueous layer, 50 to 100 μl samples were taken from each phase, and transferred to glass scintillation vials. The samples were taken to dryness, after which 10 ml of scintillation fluid (Hall & Cocking 1965) was added. Distribution coefficients were calculated as the ratio of the counts in the two phases.

Animal experiments

Adult male Wistar rats (CFHB), (Carworth, Europe) were used.

Solvent toxicity. Two rats, 70 to 100 g, were used for each solvent. One rat was given, by gavage, 0·25 ml, and the other 0·5 ml. They were observed over 8 h, and again after 24 h.

Collection of blood, urine and faeces. Groups of 5 rats weighing 152 ± 10 g were fasted overnight, but provided with free access to water. Each animal was given 0·5 ml of solution of methyltestosterone by gavage, equivalent to 4 μCi. Urine and faeces were collected over 6 days, and their radioactivities measured. Urine was added directly to the scintillation fluid, but faeces were first solubilized with 2 M methanolic potassium hydroxide before addition to the scintillation fluid (Nicholls & Orton 1972).

In preliminary experiments, 20 μl blood samples were taken from the tail, at hourly intervals for 9 h, and their activities measured by scintillation counting. When solvent blends were investigated, 20 rats, each 70 to 80 g, were selected for each solution and fasted overnight, with free access to 5% w/v glucose instead of water, to reduce copraphagy. 0·5 ml of solution containing 3 μCi of [^{3}H]methyltestosterone and 1 mg of carrier was given intragastrically to each rat. Blood was collected by cardiac puncture from groups of 5 rats, under chloroform anaesthesia, at 5, 15 min, 1 and 3 h after dosing. Plasma was separated by centrifuging at 3000 rev min^{-1} for 10 min, and aliquots added to scintillation fluid for counting.

Gut. After blood sampling, the animals were killed under chloroform, the entire gastrointestinal tract removed and tied off at the oesophagus and rectum and, after superficial fat and mesentery were removed, homogenized in a few ml of water containing 400 μl of polysorbate 80. The homogenates were made up to 20 ml, and 50 μl aliquots taken for counting, as before.

Collection of bile. Six rats, 300 ± 10 g, were used for each solution, and pretreated as before. They were anaesthetized with 6 ml kg^{-1} of 25% urethane, given intramuscularly, and their bile ducts cannulated with polythene tubing. The cannula of one rat was inserted into the duodenum of another, which was given 0·5 ml of solution containing 5 μCi[^{3}H]methyltestosterone and 1 mg carrier, intragastrically. Bile was collected from the treated rat at 15 min intervals over 3 h. Three 10 μl samples of each were taken and freeze dried before counting.

Establishment of dose level. Male rats weighing 130 ± 30 g were castrated under ether anesthesia, and 14 days later, given by gavage either 0·3 or 1·0 mg methyltestosterone, dissolved in decanol. Control rats received solvent only. Three rats at each dose level, plus 3 control rats, were killed on days 15, 18 and 25, and ventral prostate glands and seminal vesicles dissected, dried and weighed. Increases over control in seminal vesicle weights

were observed with both doses, but 0·3 mg was insufficient to produce a significant response in prostate.

RESULTS AND DISCUSSION

The tritiated material obtained from the Radiochemical Centre had not been purified. Obvious contaminants were 17α-methylandrostanolone, 17α-methyl-17β-hydroxyandrost-1-en-3-one and the starting material. Eleven different chromatography systems were examined for separation of methyltestosterone. [4,5-^{3}H]-17α-Methyl-17β-hydroxyandrost-1-en-3-one could not be obtained commercially for comparison, but its absence from the tritiated mixture could be assumed, since in none of the chromatography systems used was a radioactive spot detected which could not be accounted for. Quantitative separation, using the best chromatographic system revealed that the tritiated material consisted of 35% methyltestosterone and 65% methylandrostanolone.

As distribution coefficients frequently vary with concentration, because of complex formation, it is usual to determine them using several concentrations of solute, and extrapolating the results back to infinite dilution. By use of carrier-free solutions it was possible to obtain distribution coefficients at very low solute concentrations, which were taken as infinite dilution values.

Preliminary experiments gave results which were reproducible for a given equilibrium mixture, but showed excessive variation when different mixtures having the same stoichiometric composition were examined. The variation was attributed to tritium exchange between solute and solvents. Hawkins (1976) suggested that freeze drying removes radioactive solvent, leaving only the solute. This method was adopted in the present work, results are shown in Table 1. The standard errors indicate that variation is low when freeze drying is used. Exchange was also much reduced when a carrier was added. Biological measurements were therefore made with inactive methyltestosterone as carrier.

Choice of solvents was limited, natural products such as fixed oils were rejected because composition could not be controlled, and esters were avoided because of possible complications arising from in vivo hydrolysis. Furthermore, the liquid had to be a reasonably good solvent for methyltestosterone, and be sufficiently insoluble in water to make distribution possible. With such criteria, the list of possible solvents was reduced to saturated hydrocarbons, fatty alcohols and halogenated hydrocarbons. A final restriction was acute toxicity, which had to be minimal at the doses to be employed in the rat. However, possible chronic toxicity problems were ignored, since the experiments were short term. 0·25 ml, half the proposed dose of solution, gave rise to disorientation on oral administration of chloroform, and carbon tetrachloride caused immediate death. The same volume of either tetralin or decalin caused hyperactivity in the rats. The C_7 to C_{11} n-alkanes, n-hexadecane and the C_5 to C_{10} fatty alcohols produced no overtly adverse effect, even with 0·5 ml, apart from a slight initial sedation after administration of 0·5 ml of n-heptanol. These paraffins and alcohols were therefore selected for further study.

Table 1. Distribution coefficients of [^{3}H]methyltestosterone between octane, octanol and octane-octanol blends, and water.

Solvent	Molar distribution coefficient	Number of samples	Standard error of mean ($P' = 0·01$)
Octane	5·43	10	0·03
5% Octanol 95% Octane	12·5	5	0·09
30% Octanol 70% Octane	30·1	7	0·35
Octanol	46·5	7	0·53

In preliminary experiments, urine and faeces were collected for 6 days. All of the administered tritium was excreted in the urine and faeces over this period. When the solvent was an alkane, 75 to 80% was excreted in the urine and the remainder in the faeces, but with the alcohols, 55 to 60% was eliminated in the urine. Tritium concentrations in blood drawn from the tail veins rose and fell with time over the 8 h after dosing. Alcohols gave lower values than alkanes, and the onset of the first maximum was slower. Differences within either series were too small to distinguish between individual homologues, thus for the alcohols, the highest and lowest mean blood concentrations per ml, expressed as percentage of administered dose were $3·06 \pm 0·89 \times 10^{-2}$% for octanol and $2·44 \pm 0·77 \times 10^{-2}$% for undecanol ($P' = 0·05$). However, the two series were significantly different, the alkanes gave a mean blood concentration of $5·42 \pm 0·46 \times 10^{-2}$% and the alcohols $2·82 \pm 1·08 \times 10^{-2}$%. Similar results were obtained with plasma samples taken by cardiac puncture. Subsequent comparisons were therefore made between an alkane and an alcohol, together with blends to

give intermediate results. Octanol and octane were selected, both being sufficiently volatile to be freeze dried easily, but sufficiently involatile to be stored without excessive evaporation. The solvents and blends gave a satisfactory spread of distribution coefficients, as shown in Table 1. Two solvents of the same chain length were used to minimize steric differences between them, and to reduce deviations from ideality in the solvent blends. Preliminary work established that the two solvents were miscible in all proportions.

Plasma concentrations, after administration of solutions of labelled methyltestosterone in the octane–octanol mixtures, are plotted in Fig. 1. For clarity, confidence limits are shown only for the pure octane and octanol solutions. Those for the blends were similar. There was a rapid increase in plasma tritium concentration from the solution in octane, to a value well in excess of the maximum obtained from the octanol solution. The blends occupied positions between the two. Disappearance from the gastrointestinal tract followed a similar pattern (Table 2). There was a rapid fall in gut concentration from the solution in octane, with a minimum ca 60% after 15 min. In contrast, less than 15% was absorbed from the octanol solution.

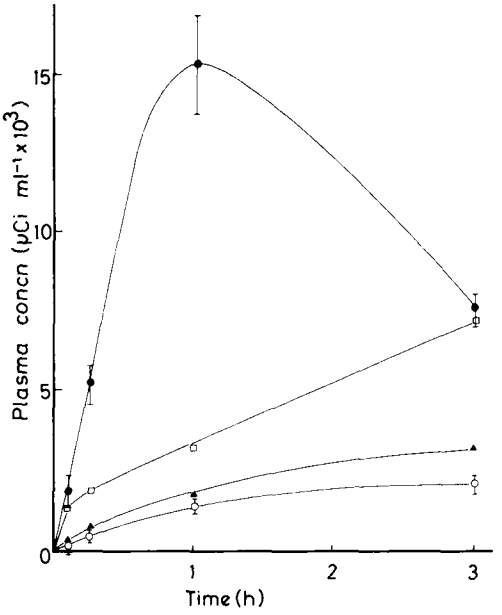

FIG. 1. Plasma tritium concentrations following oral administration of [^{3}H]methyltestosterone in solution. ● Octane. □ 5% Octanol + 95% octane. ▲ 30% Octanol + 70% octane. ○ Octanol.

Table 2. Absorption of tritium from the gastrointestinal tract following oral administration of [^{3}H]methyltestosterone solution.

Solvent	Percentage absorbed 5 min	15 min	1h	3h
Octane	21·2 (3·9)	37·4 (4·6)	36·9 (2·5)	36·7 (3·1)
Octanol 5% Octane 95%	26·6 (4·8)	31·0 (3·0)	36·1 (5·3)	41·4 (2·9)
Octanol 30% Octane 70%	27·4 (3·9)	31·9 (3·8)	34·2 (4·6)	34.6 (2·4)
Octanol	9·4 (1·8)	7·5 (2·9)	6·1 (2·1)	13·0 (1·7)

The figures in parentheses represent 95% confidence limits.

The blends gave similar profiles to the octane solution, but absorption was slower.

Fluctuation of tritium concentrations in the blood taken from the tails over 9 h was attributed to biliary recycling. Tritium concentrations in the bile were therefore examined. As cannulation of the bile duct in the test animal left the duodenum deficient in bile, which could have influenced absorption, bile from a cannulated donor animal was introduced into the duodenum of the test rat throughout the experiment. The results [octane 39·8, octanol–octane (5:95%) 37·8, (30:70%) 8·25, octanol 0·81] follow a pattern consistent with the data obtained for blood, urine and faeces, all of which suggest that the rate and extent of gastrointestinal absorption of radioactivity are greater from alkane solution than from alkanol solution.

Gastric emptying time could be sensitive to the nature of the solvent, thereby influencing the rate of absorption of methyltestosterone, but this would be apparent only during absorption and elimination. Differences were detected at completion, when all the tritium had been excreted, so that this process was not considered a complicating factor. The proposed effect of solvent on absorption is based on tritium concentrations, which are not necessarily the same as methyltestosterone concentrations. Testosterone is known to undergo gut-wall metabolism, and the process could be sensitive to the presence of an organic solvent. However, methyltestosterone is orally active because it is resistant to enzyme attack. A dose of 1 mg was chosen because it was found to produce a measurable response in indicator organ weights, and therefore could be used to obtain biological data for comparison with the results reported here. Chaudry & James (1974) found that a

1 mg dose of nandrolone decanoate was necessary to produce a measurable response in rat after intramuscular injection. The human dose is 25 mg, indicating the same twenty-five-fold ratio between the effective doses in man and rat. This high dose of methyltestosterone would be expected to saturate any possible metabolism in the gut.

The variation in tritium concentrations with changing solvent composition indicates that its availability is less from alkanol solutions than from alkane solutions. Methyltestosterone is more soluble in alkanols than in alkanes. Inverse relationships between solubility and absorption are well established in percutaneous transport studies, and it is therefore not unreasonable to speculate that the behaviour described here is controlled in some way by solubility or a related property, such as distribution. The distribution coefficients between solvent blends and water changed progressively in moving from pure octane to pure octanol, but no simple relationship between distribution coefficient and any of the in vivo results could be obtained. Nevertheless, all followed the same rank order as distribution coefficients. Armstrong et al (1979), using non-aqueous solutions of a range of aromatic acids, also failed to demonstrate a quantitative relationship with absorption from the gastrointestinal tract, although rank order correlations between their distribution coefficients and in vivo results can be detected. They suggest that the rate-determining property was the velocity of migration from the non-aqueous phase to the aqueous phase, which infers that the process of absorption occurring in the gut is in a dynamic state, and that the lipid and aqueous phases are not in equilibrium. Such a process would be influenced by solubility. This could be the mechanism responsible for the results described above.

Acknowledgements

The authors wish to acknowledge the gift of methyltestosterone from Organon Laboratories Ltd. We are grateful to the S.R.C. for an award to L.M.S.

REFERENCES

Alibrandi, A., Bruni, G., Ercoli, A., Gardi, R., Meli, A. (1960) Endocrinology 66: 13–18

Armstrong, N. A., James, K. C., Wong, C. K. (1979) J. Pharm. Pharmacol 31: 657–662.

Bruni, G., Galletti, F., Ercoli, A. (1966) Eur. J. Steroids 1: 29–36

Bruni, G., Galletti, F. (1970) Steroidologia 1: 89–93

Bruni, G., Galletti, F. (1973) Boll. Chim. Farm. 112: 110–115

Bush, I. E. (1961) The Chromatography of Steroids, London, Pergamon

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

Faint, P. D., King, G. H. (1970) Paper presented at a technical colloquium on 'Automated Analysis in the Pharmaceutical Industry', London, April 23rd

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

Hawkins, D. R. (1976) Chem. Br. 12: 379–383

Nicholls, P. J., Orton, T. C. (1972) Br. J. Pharmacol. 45: 48–59

Quincey, R. V., Gray, C. H. (1966) J. Endocrinol. 35: 121–122