The Pharmacokinetics of Trilostane and Ketotrilostane in an Interconverting System in the Rat

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The pharmacokinetics of trilostane and one of its metabolites ketotrilostane are described and characterized in the rat following the separate intravenous administration of trilostane and ketotrilostane. It was noted during these studies that the parent compound and its metabolite undergo metabolic interconversion—trilostane producing ketotrilostane and ketotrilostane generating trilostane. This result means that trilostane is conserved in the body by interconversion—being metabolized to ketotrilostane and then subsequently back to the "parent" drug, trilostane.

KEY WORDS: trilostane; ketotrilostane; reversible metabolism; pharmacokinetics; metabolic interconversion.

INTRODUCTION

Since the first publication discussing metabolite kinetics (1) in 1963, this field of research has been the subject of several extensive reviews (2–4). The importance of the pharmacokinetics of a metabolite can lie in two areas. When the metabolite is active (either a pharmacological activity similar to that of the parent or a toxic effect), its time course will be of relevance, and when the pharmacokinetics of the parent compound cannot be determined, the pharmacokinetics of the metabolite may be ascertained and used as a reflection of the fate of the parent compound. Drug therapy may be easily influenced by the pharmacokinetics of metabolite production since drug metabolism usually acts as a route of elimination. The rate of formation of a metabolite from an active drug and the elimination of the metabolite with a different degree of activity will hence determine the overall extent and duration of pharmacological activity.

Many of the parameters which are used to describe metabolite disposition can be obtained only after intravenous administration of the preformed metabolite and its subsequent determination in blood or plasma. Administration of the parent compound and measurement of the metabolite will enable the calculation of the AUC of the metabolite and the determination of the elimination rate constant ($k_{(m)}$) of that metabolite only if its rate of elimination is slower than its rate of formation. However, following the intravenous and oral administration of the metabolite, all the necessary pharmacokinetic parameters required to describe the absorption and disposition of the parent compound may also be calculated for the metabolite (3).

Trilostane ([4α, 5α, 17β]-4,5-epoxy-3,17-dihydroxyandrost-2-ene-2-carbonitrile) (Fig. 1a) is a synthetic steroid which has been shown to produce reversible inhibition of 3β-hydroxysteroid dehydrogenase in laboratory animals (5). In man, the drug is beneficial in the treatment of Conn's and Cushing's syndrome (6–8) and has recently been shown to be useful in estrogen- and progesterone-positive breast cancer in postmenopausal women (9). The variability exhibited in systemic levels of trilostane following oral administration (10) is possibly due in part to suboptimal absorption owing to its low water solubility.

Five metabolites of trilostane have been isolated (11) following the oral administration of trilostane to a group of rats. These authors proposed the metabolic pathways shown in Fig. 2 and suggested that since ketotrilostane ([4α, 5α]-4,5-epoxy-3-hydroxy-17-oxoandrost-2-ene-2-carbonitrile) (Fig. 1b) was the major metabolite in bile, it may also be the first intermediate in the metabolism of trilostane. This was supported by the knowledge that the oxidation of C-17 hydroxyl groups is common in androstanes (12). This metabolic pathway implied that trilostane and ketotrilostane could undergo metabolic interconversion, although no experimental evidence was presented to support this hypothesis, and no previous work had been performed to characterize the pharmacokinetics of ketotrilostane in rat or man.

Ketotrilostane is itself a pharmacologically active compound, and its pharmacokinetics were therefore of interest. The parent compound, trilostane, is a 17β-hydroxysteroid. The elimination of many steroids, including 17β-hydroxy steroids, is affected by reversible metabolism. 17β-Estradiol is reversibly metabolised to its 17-keto metabolite oestrone, and testosterone also undergoes metabolic interconversion through 17β-hydroxy oxidoreductase (13). Since reversible metabolism affects the fate of drugs in the body, we decided to ascertain whether trilostane and ketotrilostane underwent metabolic interconversion.

MATERIALS AND METHODS

Chemicals and Reagents

Trilostane and ketotrilostane were generously supplied by Sterling Research Group, Alnwick, U.K. Pentobarbitone was purchased from Evans, U.K. All other chemicals were from Sigma, Poole, U.K. All materials were used as received.

Preparation of Drug Solution

Trilostane and ketotrilostane solutions were comprised of either trilostane (500 mg) or ketotrilostane (500 mg), polyethylene glycol 400 (7.5 ml), 96% (w/w) ethanol (5 ml), and polyvinyl pyrrolidone 40,000 (200 mg). The solution was adjusted to pH 9.3 ± 0.1 with 10% (w/v) sodium hydroxide and the volume made up to 25 ml with distilled water.

Pharmacokinetic Studies in Rats

Male Wistar rats weighing 350 to 370 g were used for the study. They were fed standard rat chow with water *ad libitum* and maintained on a 12-hr cycle of day and night. Anesthesia was induced with pentobarbitone (75 mg/kg) administered intraperitoneally. Additional pentobarbitone was administered as and when required to maintain anesthesia. All

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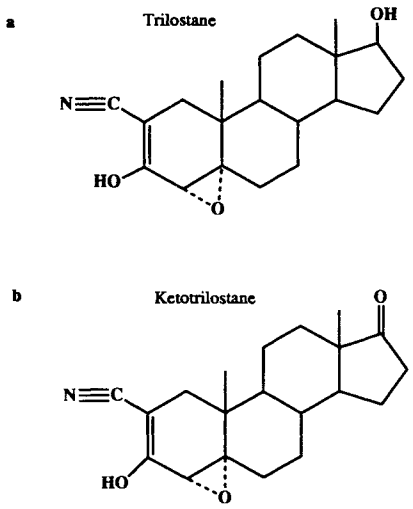


Fig. 1. The structure of (a) trilostane and (b) ketotrilostane.

rats underwent tracheotomy and jugular vein, and carotid artery cannulation. The drug solutions were intravenously administered to two groups of rats (n = 4) at a dose of 40 mg/kg. Blood samples (200 µl) were taken at the following times after drug administration: 0, 1, 5, 10, 20, 30, 60, 90, 150, and 240 min. After each blood sample was taken, 200 µl of saline was infused intravenously.

Analysis of Samples

The blood sample was centrifuged to yield 100 µl of plasma. The plasma concentrations of trilostane and ketotrilostane were determined using a previously described reverse-phase high-performance liquid chromatography (HPLC) assay method (14).

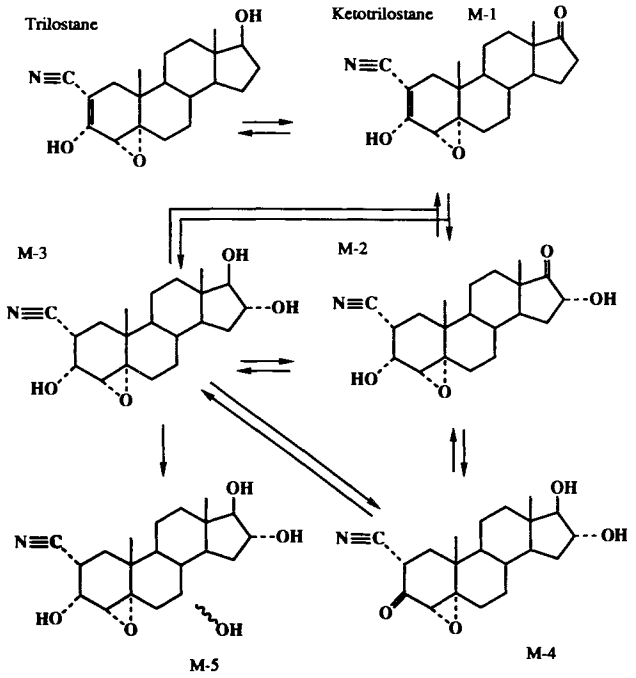


Fig. 2. The metabolic pathway of trilostane proposed by Mori *et al.* (1981).

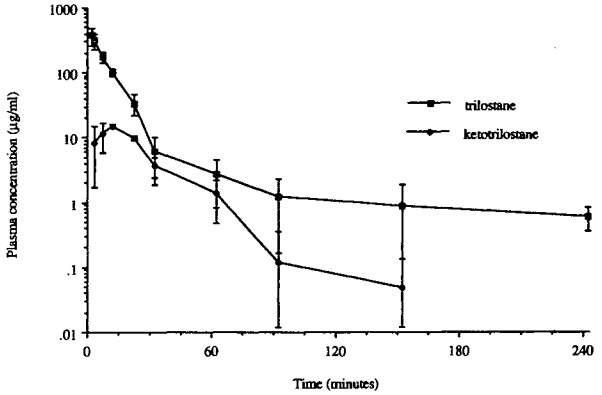


Fig. 3. Mean plasma concentrations ± SD of trilostane and ketotrilostane following intravenous administration of 40 mg/kg of the parent compound trilostane.

Pharmacokinetic Analysis

The pharmacokinetic parameters for trilostane and ketotrilostane were determined as follows: after intravenous bolus injection the initial plasma concentration and the terminal slope were estimated by fitting either biexponential or triexponential equations to the plasma concentration–time curves using the nonlinear least-squares regression program PC NONLIN. The area under the plasma concentration-versus-time curve (AUC) for both compounds was calculated using the trapezoid approximation using the nonlinear least-squares terminal slope for extrapolation to time infinity. The first moment of the plasma concentration-versus-time curve (AUMC) was calculated similarly after multiplying each plasma concentration by its time. The other pharmacokinetic parameters which describe the interconverting system were calculated as described previously (13).

RESULTS AND DISCUSSION

The mean plasma concentrations ± SD of trilostane and ketotrilostane following an intravenous dose of 40 mg/kg of trilostane are shown in Fig. 3. The decline in plasma concentration of trilostane follows a triexponential decrease; the $t_{1/2}$ α was rapid, with a mean half-life of 4.01 ± 0.77 min, and the $t_{1/2}$ β was longer, with a mean half-life of 19.2 ± 7.2 min

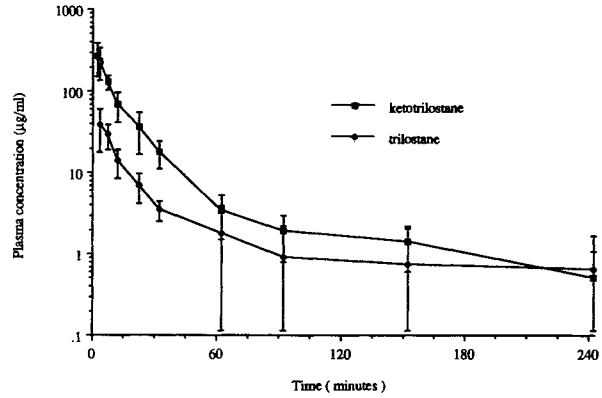


Fig. 4. Mean plasma concentrations ± SD of ketotrilostane and trilostane following intravenous administration of 40 mg/kg of the metabolite ketotrilostane.

Table I. Pharmacokinetic Parameters, Obtained Using Nonlinear Curve Fitting, for Trilostane and Ketotrilostane After Their Respective Administration to Rats (40 mg/kg)

Parameter	Trilostane, $n = 4$ (triexponential)	Ketotrilostane	
		$n = 2$ (triexponential)	$n = 2$ (biexponential)
A (μg/ml)	348 ± 56	197 ± 33	224 ± 61
B (μg/ml)	14.7 ± 13.3	32.9 ± 3.0	1.34 ± 0.51
C (μg/ml)	1.49 ± 1.06	2.27 ± 1.10	—
α (/min)	0.177 ± 0.034	0.162 ± 0.162	0.088 ± 0.013
β (/min)	0.040 ± 0.016	0.045 ± 0.004	0.0034 ± 0.0009
γ (/min)	0.0041 ± 0.0021	0.0051 ± 0.0021	—
Terminal half-life (min)	219 ± 145 ($n = 4$)	178 ± 57 ($n = 4$)	

and an elimination half-life of 219 ± 145 min. The metabolite, ketotrilostane, was very rapidly formed, with peak concentrations occurring in some animals within 5 min.

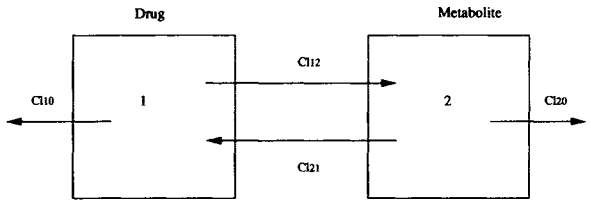
The mean plasma concentrations ± SD of ketotrilostane and trilostane against time following intravenous administration of 40 mg/kg of ketotrilostane are shown in Fig. 4. The decrease in mean ketotrilostane plasma concentration illustrated in Fig. 4 appears to follow a triexponential decrease, although it is less marked than that of trilostane as illustrated in Fig. 3. Trilostane is rapidly formed after intravenous administration of ketotrilostane, the plasma concentration decreasing in parallel with ketotrilostane after 60 min and continuing in parallel over the sampling period.

Models to describe the data were fitted to the individual plasma concentrations for the 40-mg/kg doses of trilostane and ketotrilostane using the nonlinear least-squares regression program PC NONLIN (15). For trilostane a triexponential model was fitted to all four sets of data. A triexponential model was fitted to only two of the four sets of data for ketotrilostane; a biexponential model was fitted to the other two sets of data. The parameters calculated and the values obtained using PC NONLIN are shown in Table I. Since models could not be fitted satisfactorily to the "metabolite" data obtained after administration of the "parent" compound, AUC values for trilostane and ketotrilostane after both administrations were calculated using the trapezoid approximation for the purpose of maintaining consistency.

It is not uncommon for a lack of clarity as to whether the distribution/elimination of the drug is best described by a bi- or triexponential function. Following intravenous administration of digoxin, it was demonstrated that a semilogarithmic plot of the blood concentrations could be separated into two or three exponential phases depending on sampling times and the graphical or computational technique employed (16–18).

It is evident from the results in Figs. 3 and 4 that interconversion between the drug trilostane and its metabolite ketotrilostane is occurring. It can also be seen that the metabolic interconversion of trilostane and ketotrilostane therefore serves to conserve drug and its active metabolite. Hence, instead of ketotrilostane being metabolized to the inactive metabolites M2, M3, M4, and M5 as illustrated in Fig. 2, it may be reconverted to its pharmacologically active parent compound, which in turn will again be metabolized to ketotrilostane. This observation is important since it necessitates the use of a model to describe the interconversion system, as illustrated in Fig. 5. The equations used to calculate the pharmacokinetic parameters have been described previously (13) and are repeated in the Appendix.

The values obtained for the pharmacokinetic parameters calculated after the intravenous administration of 40 mg/kg of trilostane and those obtained after the intravenous administration of 40 mg/kg of ketotrilostane are shown in Table II. There was no significant difference for the mean AUC values of trilostane and ketotrilostane after administration of trilostane and ketotrilostane, respectively, but the mean AUC value of trilostane after administration of ketotrilostane (312 ± 87 μg/ml · min) was significantly less than the mean AUC value of ketotrilostane after administration of trilostane (629 ± 224 μg/ml · min). The value of Cl_{12} is greater than Cl_{21}. This indicates that the interconversion process favors the metabolism of ketotrilostane to trilostane. The elimination clearances (Cl_{10} and Cl_{20}) and hence the irreversible elimination of both trilostane and ketotrilostane are similar in this system. The elimination capacity or "real" clearance operating on each compound is the sum of the respective clearance terms operating on the drug and metabolite, respectively. Hence, Cl^{p}_{real} is the arithmetic sum of Cl_{10} and Cl_{12}, whereas Cl^{m}_{real} is the sum of Cl_{20} and Cl_{21} (Fig. 5). The mean values for Cl_{real} were not significantly different for both compounds, indicating that although the interconversion favors the generation of trilostane from ketotrilostane, this is compensated by a higher elimination clearance of the parent drug. The apparent volume of distribution at steady state (V_{ss},app) is calculated using conventional moment analysis, with the values for trilostane (135 ± 53 ml) and ketotrilostane (102 ± 24 ml) not being significantly different from the values obtained, V_{ss},real. The ap-



Cl10 and Cl20 Clearance terms for the irreversible loss of drug and metabolite
Cl12 and Cl21 Clearance terms for the metabolic interconversion of drug and metabolite

Fig. 5. A diagramatic illustration of the interconverting system.

Table II. Pharmacokinetic Parameters Calculated for Trilostane and Ketotrilostane Following Intravenous Administration of 40 mg/kg of Trilostane and Ketotrilostane, Respectively[a]

Parameter	Trilostane, 40 mg/kg (n = 4)	Parameter	Ketotrilostane, 40 mg/kg (n = 4)
AUC^p_p (μg · min/ml)	2805 ± 633	AUC^m_m (μg · min/ml)	2614 ± 474
AUC^p_m (μg · min/ml)	312 ± 87	AUC^m_p (μg · min/ml)	629 ± 224
Cl_{10} (ml/min)	4.36 ± 1.00		
Cl_{20} (ml/min)	3.97 ± 0.52		
Cl_{12} (ml/min)	0.64 ± 0.38		
Cl_{21} (ml/min)	1.31 ± 0.70		
Cl^p_{real} (ml/min)	5.00 ± 1.33	Cl^m_{real} (ml/min)	5.28 ± 1.14
$V^p_{ss,app}$ (ml)	135 ± 53	$V^m_{ss,app}$ (ml)	102 ± 24
$V^p_{ss,real}$ (ml)	132 ± 52	$V^m_{ss,real}$ (ml)	97 ± 27
Sojourn time (min)	18.3 ± 8.2	Sojourn time (min)	19.2 ± 6.8

[a] AUC^p_p, AUC for trilostane after trilostane administration. AUC^p_m, AUC for ketotrilostane after trilostane administration. AUC^m_m, AUC for ketotrilostane after ketotrilostane administration. AUC^m_p, AUC for trilostane after ketotrilostane administration. Cl_{10}, Cl_{20}, Cl_{12}, Cl_{21}, $Cl_{p,real}$, $V^p_{ss,real}$, and $V^p_{ss,app}$, see text for details.

parent volume of distribution term is biased by the contribution of the interconverting system. The mean value for V_{ss},real was only slightly less for ketotrilostane (97 ± 27 ml) than for trilostane (132 ± 52 ml). In a system where reversible metabolism is operating, the mean residence time for a compound is better expressed as a "sojourn time." This parameter gives a better reflection of the turnover of the parent and metabolite in such a system. The sojourn times for both compounds studied here in the rat were not significantly different.

It was not possible to investigate the potential nonlinearity occurring in trilostane and ketotrilostane pharmacokinetics and hence in this interconverting system.

The role of reversible metabolism in pharmacokinetics has received limited attention as shown by the small number of publications dealing with this aspect of pharmacokinetics. Known examples include 17β-estradiol (19), canrenone (20,21), prednisolone (22), methylprednisolone (23), testosterone (24), and sulindac (25), all having metabolites which are capable of reverting to the parent compound.

Metabolic interconversion affects calculations in studies of drug absorption, yielding an overestimation of drug bioavailability because, instead of one source of drug input from the gut lumen, there will be an additional source of drug from the systemic interconversion of metabolite and drug. Methods for the assessment of the bioavailability of drugs which are subject to interconversion with a metabolite have been established (13,26).

APPENDIX

$$Cl_{10} = \frac{dose^p \cdot AUC^m_m - dose^m \cdot AUC^p_m}{AUC^p_p \cdot AUC^m_m - AUC^p_m \cdot AUC^m_p}$$

$$Cl_{20} = \frac{dose^m \cdot AUC^p_p - dose^p \cdot AUC^m_p}{AUC^p_p \cdot AUC^m_m - AUC^p_m \cdot AUC^m_p}$$

$$Cl_{12} = \frac{dose^m \cdot AUC^p_m}{AUC^p_p \cdot AUC^m_m - AUC^p_m \cdot AUC^m_p}$$

$$Cl_{21} = \frac{dose^p \cdot AUC^m_p}{AUC^p_p \cdot AUC^m_m - AUC^p_m \cdot AUC^m_p}$$

$$V^p_{ss,real} = \frac{dose^p \cdot (AUM^m_m)^2 \cdot AUMC^p_p - AUC^p_m \cdot AUC^m_p \cdot AUMC^m_m}{(AUC^p_p \cdot AUC^m_m)^2 - (AUC^p_m \cdot AUC^m_p)^2}$$

$$V^m_{ss,real} = \frac{dose^m \cdot (AUC^p_p)^2 \cdot AUMC^m_m - AUC^p_m \cdot AUC^p_m \cdot AUMC^p_p}{(AUC^m_m \cdot AUC^p_p)^2 - (AUC^p_m \cdot AUC^p_m)^2}$$

$$\bar{S}^p = \frac{V^p_{ss,real}}{Cl_{10} + Cl_{12}}$$

$$\bar{S}^m = \frac{V^m_{ss,real}}{Cl_{20} + Cl_{21}}$$

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