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PHARMACOKINETICS OF THE GONADOTROPHIN INHIBITOR 19β-*CIS*-ETHYLIDEN-17α-ETHYNYL-1,4,6-ANDROSTATREIN-3-ONE (Ro 6-5403) IN MAN

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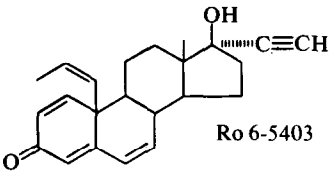
SUMMARY

A pharmacokinetic profile of the gonadotrophin inhibitor, 19β-*cis*-ethyliden-17α-ethynyl-17β-hydroxy-1,4,6-androstatrien-3-one (Ro 6-5403) was determined in two female subjects following oral and intravenous administration of tritium labelled drug. The plasma level of intact Ro 6-5403 was approximately 1 μg/100 ml at 1 h following oral administration of 2 mg of drug and had declined to about 0·2μg/100 ml after 8 h. The plasma disappearance curve after intravenous injection was consistent with a two-compartment open system model and indicated fast distribution and elimination with a half life of 86–116 min. Over 60 % of the radioactive dose was excreted in the urine within 3 days following either route of administration. Only about 1 % of the urinary radioactivity was associated with unchanged Ro 6-5403, indicating almost complete biotransformation and/or alternate routes of excretion. Investigation of the distribution of ^{3}H-activity in the urine revealed that 5 % was unconjugated, 20 % released by β-glucuronidase hydrolysis and 25 % by solvolysis.

INTRODUCTION

19β-*cis*-ethyliden-17α-ethynyl-17β-hydroxy-1,4,6-androstatrien-3-one (Ro 6-5403) represents a hitherto undescribed class of gonadotrophin inhibitors of marked oral potency with extremely weak estrogen-like characteristics which have been shown by *in vitro* metabolic studies not to be the results of A-ring aromatization.



Ro 6-5403

Animal data suggest that its properties are such that it could be used to set the hypothalamic–hypophysial-ovarian system largely at rest, thus producing infertility combined with amenorrhea, without vaginal mucosal atrophy.

In rats, Ro 6-5403 brings about not only reduction of the hypophysial gonadotrophin contents and presumably consequent inhibition of growth of gonadotrophin-dependent peripheral organs, but also exhibits marked antagonism of the stimulation of such organs induced in hypophysectomized animals by PMSG and HCG. Its gonadotrophin inhibiting properties may therefore be regarded not only as central but also as peripheral in character. In addition, Ro 6-5403 inhibits the

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stimulatory effect of progesterone on the estrogen-primed endometrium of the juvenile rabbit. Although a chemical derivative of ethisterone (17α-ethynyl-17β-hydroxy-4-androsten-3-one), an overtly androgenic substance, Ro 6-5403 lacks even the weaker androgenicity of norethisterone (17α-ethynyl-17β-hydroxy-4-estren-3-one), although dose for dose it appears a more potent gonadotrophin antagonist than the latter substance. Ro 6-5403 appears to possess weak inherent estrogenicity, showing ca. 500 times less activity in the Allen–Doisy test than that of the reference substance ethynylestradiol (3β,17β-dihydroxy-17α-ethynyl-1,3,5(10)-estratriene). When tested orally against ethynylestradiol as an ovulation inhibitor, it proved only about 40 times weaker on a weight basis [1].

In view of these findings, Ro 6-5403 is being investigated clinically for its gonadotrophin inhibiting properties in man.

The present investigation was carried out in order to obtain information regarding the pharmacokinetics, bioavailability and excretion of Ro 6-5403 in female subjects.

EXPERIMENTAL

Materials

Chromatography

Thin layer chromatography (t.l.c) was carried out on pre-coated plates of silica gel F254 (Merck AG). Paper chromatography employed Whatman No. 1 paper. The following chromatographic solvent systems were used: (A) methylene chloride: acetone (4:1, v/v), (B) benzene: ethylacetate (3:2, v/v), (C) isooctane: benzene: methanol: water (7:3:7:3, by vol). Steroids were eluted with methylene chloride: methanol (9:1, v/v).

Measurement of radioactivity

Tritium was determined in a Packard Model 3375 liquid scintillation spectrometer. Aqueous samples were counted in Insta-Gel (Packard) and all other samples in toluene containing 5 g PPO and 0·3 g dimethyl-POPOP/Liter. The efficiency for counting ^{3}H in the toluene scintillator was 52 % for unquenched samples. Quenching was corrected by use of the automatic external standard and results expressed in d.p.m. Sufficient counts were accumulated to afford a statistical error of $\pm$ 3 %.

Radioactive materials

[19-^{3}H]-19β-*cis*-ethyliden-17α-ethynyl-17β-hydroxy-1,4,6-androstatrien-3-one (19-^{3}H-Ro 6-5403) was supplied by F. Hoffmann-La Roche & Co. AG., Basle Switzerland as crystalline tracer (specific activity: 120μCi/mg) and 2 mg tablets (specific activity: 22·5 μCi/mg). The crystalline material was chromatographed on silica gel with Systems A and B and its radiochemical purity ascertained by crystallisation to constant specific activity with carrier.

Methods

Subjects

Two normal female volunteers (N.D., age 33, 67 kg and E.M., age 22, 73 kg) participated in this study.

Administration of 19-^{3}H-Ro 6-5403

The tracer, as a solution in 1 ml ethanol and 9 ml isotonic saline was administered by rapid intravenous injection into an arm vein. Subjects N. D. and E. M. received $4{\cdot}44 \times 10^7$ (196 μg) and $8{\cdot}24 \times 10^7$ d.p.m. (310 μg) of 19-^{3}H-Ro 6-5403 respectively. Some weeks later Subject E.M. received a single 2 mg tablet (45 μCi) of 19-^{3}H-Ro 6-5403 orally.

Plasma

At the times indicated in the figures, 5 ml of blood was taken, treated with heparin, the plasma immediately separated by centrifugation and stored at $-20°$.

Urine

Collected at 24 h intervals for 3 days and stored at 4°.

Total ^{3}H-*activity in plasma and urine*

Duplicate 0·1 ml aliquots of plasma and 0·5 ml aliquots of urine were assayed.

Measurement of Intact Ro 6-5403

Plasma

A measured volume of plasma was diluted with 100 μg of unlabelled Ro 6-5403 as carrier. The mixture was extracted twice with 5 ml of ether and the combined extracts washed with water. After drying with anhydrous sodium sulfate the solvent was evaporated and the residue applied with methylene chloride to a silica gel plate as a 4 cm band. Following chromatography with System A, the U.V. absorbing band of the carrier Ro 6-5403 was eluted and consecutively rechromatographed on paper and silica gel with Systems C and B respectively. The final TLC eluate was quantitated by measuring the optical density at 304 nm in methanol against a standard solution of Ro 6-5403. All of the sample was then assayed for ^{3}H content and the activity corrected for the original plasma volume and the recovery of the carrier which averaged 52 %.

Urine

A 50 ml aliquot of each collection was diluted with 200 μg of carrier Ro 6-5403 and carried through the same procedure as the plasma on a slightly larger scale. Aliquots of the initial ether extract were counted to determine total *unconjugated* ^{3}H-activity.

The remaining aqueous phase was put under vacuum at 50° in a rotary evaporator to remove traces of ether and then buffered at pH5 with acetate buffer (0·1 M). β-Glucuronidase was added to give a concentration of 1000 units/ml and the mixture incubated at 37° for 2 days. An ether extract was prepared in the usual way to yield a *glucuronide fraction.* The aqueous phase was now made 0·5 M with pyridinium sulfate and extracted with chloroform. The residue obtained after solvent evaporation was subjected to solvolysis in dioxane at 37° overnight (2). An ether extract was prepared to yield a *sulfate fraction.*

RESULTS AND DISCUSSION

Pharmacokinetic evaluation

The plasma levels of intact Ro 6-5403 following intravenous administration to two female subjects are shown in Figs. 1 and 2. The plasma level-time curve

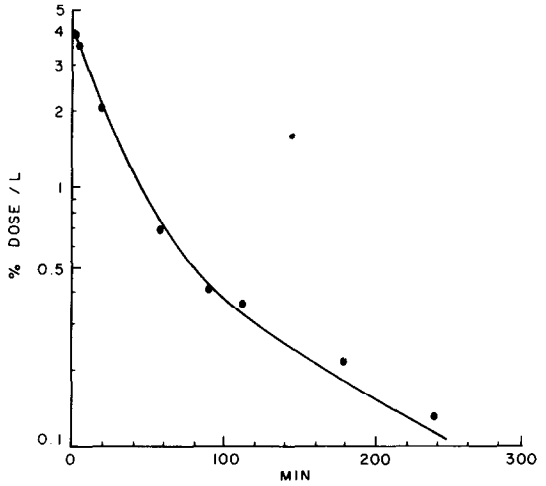


Fig. 1. Computer-simulated curve and experimental data points of plasma concentration of intact Ro 6-5403 following i.v. administration of 19-^{3}H-Ro 6-5403 ($4{\cdot}44 \times 10^7$ d.p.m., 196 μg) to Subject N.D.

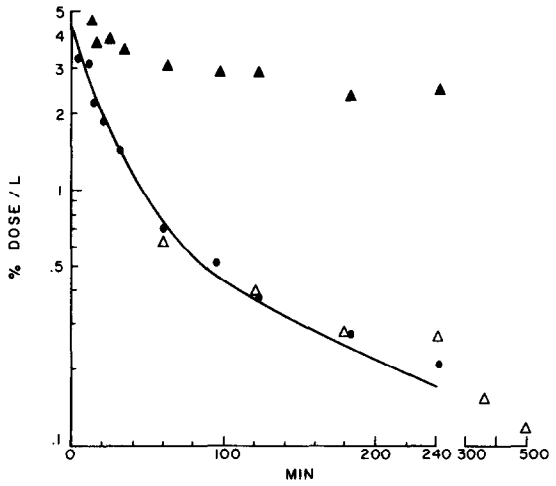
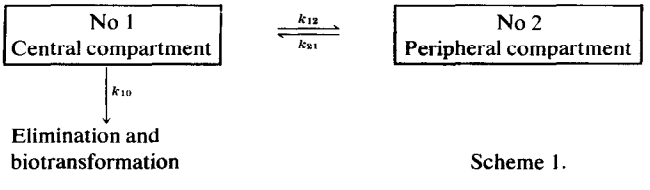


Fig. 2. Plasma concentrations of total ^{3}H-activity (▲) and intact Ro 6-5403 (●) with computer-simulated curve after i.v. administration of 19-^{3}H-Ro 6-5403 ($8{\cdot}24 \times 10^7$ d.p.m., 310 μg) to Subject E.M. Also the concentrations of intact Ro 6-5403 following oral administration (△) of Ro 6-5403 ($1{\cdot}0 \times 10^8$ d.p.m; 2 mg)

obtained was bi-exponential, indicating a minimal two-compartment open system model (Scheme 1) for analysis of the data [3].



Scheme 1.

Solution of the linear differential equations associated with the two-compartment model yields the following integrated equation for a bi-exponential process [3, 4]:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

where C_p is the concentration of the drug in the plasma, A is the zero-hour intercept and α the rate constant of the fast disposition phase and B and β are the corresponding parameters of the slow disposition phase. Employing preliminary graphical estimates [5, 6] of A, B, α and β as input, the plasma data were fitted to Eq. (1) by means of a NON-LIN digital computer programme which performs an iterative non-linear regression analysis on the entire plasma level-time curve. The various pharmacokinetic parameters associated with the model are shown in Table 1.

The initial fast distribution phase, α, has a half-life of about 16 min while the overall elimination rate constant, β, has a corresponding half-life of 86–116 min. The latter reflects the apparent rate of elimination of the drug from the body as a function of all the processes involved at pseudo-distribution equilibrium.

The individual rate constants associated with the model are calculable from the parameters of Eq. (1) [3, 5]. The k_{12} and k_{21} represent respectively, the rate of distribution into and out of the peripheral compartment while k_{10} reflects the sum of the simultaneous processes of biotransformation and excretion. Although no specific physiologic counterpart can be assigned to either compartment it is apparent that Ro 6-5403 enters and leaves the peripheral compartment with equal ease. The transfer is assumed to occur by a simple first order diffusion process.

Table 1. Pharmacokinetic parameters describing the physiological disposition of Ro 6-5403 following i.v. adminstration to man in terms of a two-compartment open system model

General Equation	$C_p = Ae^{-\alpha t} + Be^{-\beta t}$ Subject N.D.	Subject E.M.
A, % dose/L	3·545	3·280
B, % dose/L	0·775	0·712
α, min^{-1}	0·046	0·041
$0{\cdot}693/\alpha$,* min	15·09	16·81
β, min^{-1}	0·008	0·006
$0{\cdot}693/\beta$, min	85·7	116·0
Rate Constants		
k_{12} min^{-1}	0·0144	0·0152
k_{21} min^{-1}	0·0149	0·0122
k_{10} min^{-1}	0·0248	0·0196
Volumes of Distribution		
V_c L	23·15	25·05
$(V_d)_\beta$ L	71·76	81·84
Model Independent Parameters		
Area under plasma concentration-time curve, % dose/L × min	172·7	198·7
MCR L/day	835	730
ϕ, %	64	58

* 0·693/constant = half life.

The volume of distribution of the central compartment, V_c, equals the dose injected (100 %) divided by the plasma concentration of Ro 6-5403 at $t = 0$ [7–9], hence

$$V_c = 100/(A + B). \tag{2}$$

In both subjects, V_c is greater than the plasma volume, corresponding to 34% of their body weight. Such an estimate is not unusual for a drug of small molecular size where the blood plasma does not behave as a discernible compartment. Mixing in the plasma is not instantaneous and may require a few circulations to complete the distribution of the injected bolus. Since diffusion and filtration of small molecules out of the capillary beds is extremely rapid, the drug has already penetrated into a much larger volume prior to initial sampling.

Gibaldi *et al.* [10] have defined a proportionality constant, $(V_d)_\beta$, for multi-compartment systems to relate drug concentration in the plasma (C_p) to the total amount of drug in the body at any time after the attainment of pseudo-distribution equilibrium (i.e. during the terminal exponential phase of drug elimination) such that:

$$(V_d)_\beta = V_c/f_c \tag{3}$$

where f_c is the constant fraction of drug in the body that is present in the central compartment during pseudo-distribution equilibrium and independent of time. For the two-compartment open model (11):

$$f_c = \beta/k_{10}. \tag{4}$$

Thus the volume of distribution at pseudo-distribution equilibrium, $(V_d)_\beta$, multiplied by C_p provides an estimate of the total amount of drug in the body at all times following the initial distribution phase. In subjects N.D. and E.M., the total volumes of distribution, $(V_d)_\beta$ were calculated to be 107 and 110 % of body weight suggesting significant tissue uptake and possible localization of Ro 6-5403.

Gurpide and Mann [12] have shown that a number of useful kinetic parameters may be calculated which are independent of the particular model chosen. The metabolic clearance rate (MCR), which may be defined as the ratio of the rate of irreversible removal of the circulating Ro 6-5403 to its concentration in peripheral plasma was calculated [12] to be 730 to 835 L/day by dividing the dose by the area under the plasma concentration-time curve. If C_p is expressed as % of injected dose/L of plasma, then:

$$\text{MCR} = 100/\left(\frac{A}{\alpha} + \frac{B}{\beta}\right). \tag{5}$$

As pointed out by Gurpide [13], MCR is a useful index of metabolism over a wide range of dosage levels. The fraction of the total amount of Ro 6-5403 leaving the circulation which is lost irreversibly (ϕ) is also a model-independent parameter and may be calculated from the shape of the plasma dissappearance curve (12) where:

$$\phi = \frac{(A + B)^2}{(A\alpha + B\beta)\left(\frac{A}{\alpha} + \frac{B}{\beta}\right)}. \tag{6}$$

It can be seen from Table 1 that around 60% of the circulating Ro 6-5403 is lost irreversibly.

Examination of the total ^{3}H-activity levels in the plasma of Subject E.M. (Fig. 2.), which include intact drug and its metabolites shows that these levels decline with a half-life of 6 h.

After i.v. administration, 63–66% of the radioactive dose is excreted in the urine within 3 days (Table 2) indicating the possibility of alternate routes of excretion and/or loss of the tritium label from position C-19. Less than 1% of the urinary radioactivity was found to be unchanged Ro 6-5403 which demonstrates that the drug is almost completely biotransformed. Such metabolism includes conjugation with both glucuronic and sulfuric acid.

Biopharmaceutical evaluation

Following oral adminstration of a 2 mg tablet of 19-^{3}H-Ro 6-5403 to Subject E.M. a plasma concentration of 1·24 μg/100 ml was reached after 1 h and had declined to 0·23 μg/100 ml after 8 h (Fig. 2).

Two factors must be considered when assessing the availability of orally

Table 2. Excretion and distribution of ^{3}H-activity in urine following i.v. and oral administration of 19-^{3}H-Ro 6-5403 to man

	Subject N.D.	Subject E.M. (% Dose)	
Total ^{3}H-activity	i.v.	i.v.	Oral
Day 1	51	47	45·1
Day 2	10	15	12·2
Day 3	2	5	3·7
Total	63	67	61·0
Intact Ro 6-5043		% Urinary ^{3}H	
Day 1	0·88	0·90	0·50
Day 2	0·13	0·17	0·20
Day 3	0·23	0·13	0·27
Unconjugated			
Day 1	8·6	8·0	5·0
Day 2	2·3	3·9	2·0
Day 3	3·4	4·5	2·0
Glucuronides			
Day 1	26·9	28·7	37·0
Day 2	17·1	21·5	—
Day 3	15·8	22·6	—
Sulfates			
Day 1	20·0	18·5	31·0
Day 2	28·6	26	—
Day 3	29·3	26	—

administered drug: (a) overall availability of the administered dose and (b) availability of the dose as intact drug. It should be noted that following oral administration the drug must pass through the liver prior to reaching the general circulation. In the case of an extensively metabolised steroid like Ro 6-5403, this "first-pass" through the liver may reduce the amount of intact drug available. Such a phenomenon could be critical if drug activity depends on the concentration of intact drug reaching the general circulation.

In the case of Ro 6-5403, it would appear that almost all of the oral dose is absorbed when one compares the total amount of ^{3}H-activity excreted in the urine (61%) with that excreted after i.v. injection (67%); the latter being equivalent to 100% absorption (Table 2).

An assessment of the availability of intact Ro 6-5403 may be obtained by comparing the areas under the intact drug plasma level curves after both routes of administration, (Fig. 2). When both sets of data are expressed as % dose/L of plasma the area under the oral curve is about 80% of that seen after i.v. injection. Thus it appears that although the dose is completely absorbed only 80% reaches the general circulation as intact Ro 6-5403, the remainder having been biotransformed in the gastrointestinal tract and/or during its "first-pass" through the liver.

The distribution of ^{3}H-activity in the urine is similar to that found after i.v. injection (Table 2). Less than 1% is present as intact Ro 6-5403 with the major portion (greater than 60%) being excreted in a conjugated form.

Unfortunately it has not been possible in these studies to determine whether Ro 6-5403 undergoes A-ring aromatization *in vivo* since the tritium label at position C-19 would be lost. Phenolic fractions of the urine have been obtained but found to contain negligible amounts of radioactivity.

Work is at present underway on the preparative isolation of the urinary metabolites and will be the subject of a future communication.

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