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Serum kinetics of the anti-cancer agent 4-hydroxyandrostenedione in the rat

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Summary. A previously described radioimmunoassay (RIA) method for the measurement of 4-hydroxyandrostenedione (4-OHA) was used to investigate the serum drug levels attained after a single oral dose in male and female rats. Marked variability of serum drug concentrations and their time course were evident in male animals at all dose levels. In the female rat, in contrast, serum 4-OHA showed fewer individual differences, rose more rapidly and was sustained at substantially higher concentrations. In all animals, 4-OHA appeared in the serum within 0.5 h following the oral dose and persisted for at least 48 h. Doubling the dose from 8 mg/kg produced a disproportionately large elevation in serum drug levels, but a further increase to 32 mg/kg did not further increase serum levels.

Introduction

One of the important areas in the endocrine treatment of breast cancer is the development of aromatase inhibitors [1, 4, 7]. Of such compounds, 4-hydroxyandrostenedione (4-OHA) has been found to be clinically effective, but little is thus far known about its disposition in the body, in particular, about the relationship between the dose regimen and the resulting serum drug levels.

We previously reported the production of specific antisera to 4-OHA [8] and, more recently, described the first direct radioimmunoassay (RIA) procedure for the drug [10]. This assay was used in the present study to investigate serum 4-OHA levels in the rat and the variation in serum drug concentrations between the sexes and the different doses given.

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Materials and methods

Animals and drug administration. Wistar albino rats of both sexes, aged between 45 and 50 days and weighing between 200 and 250 g, were fed ad libitum. Three groups of male rats and one group of females, with six animals in each group, were used in the study. Each group of male rats received an oral dose of either 8, 16 or 32 mg/kg 4-OHA, and the female group received only 16 mg/kg. The drug was dissolved in 78% polyethylene glycol 400 solution made up in deionized water. The volume of drug solution given varied between 0.5 and 1.5 ml, depending on the dose level used. Blood samples collected from the tail vein at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 24, 32 and 48 h were allowed to clot, and the serum was stored at −20° C.

4-OHA assay. Serum 4-OHA was determined by a direct RIA method previously described [9, 10]. The antiserum was raised against a 4-hydroxytestosterone-17-hemisuccinate-ovalbumin conjugate in the sheep. The assay has a sensitivity of 82 pmol/l and a limit of detection of 0.82 nmol/l. Between-assay variation is 6% at 33 nmol/l and within-assay variation is 4.2% at 3.3 nmol/l and 7.8% at 6.6 nmol/l.

Data analysis. The area under the serum 4-OHA concentration/time curve (AUC) was calculated from 0 to 48-h values. AUC, peak serum concentration (C_{max}) and time to peak (T_{max}) values were compared between the various groups of rats.

Results

Substantial levels of 4-OHA were present in the serum at 0.5 h post-dose in all animal groups; at 48 h after 4-OHA, the drug was still detectable in serum, but only in the group receiving 16 mg/kg; however, by 72 h it had disappeared from the circulation. Table 1 shows the mean serum 4-OHA concentration found in four groups of six rats each, that were given oral doses of 8, 16 and 32 mg/kg.

Large individual variations in plasma 4-OHA, both in concentration and in the time-course, were noted in all male animals, irrespective of the dose (Table 1). Drug levels fluctuated in a markedly irregular fashion, resulting in, for example, mean 4-OHA values of 2.4–6.3 nmol for 0.5–7 h following an oral dose of 8 mg/kg. Corresponding ranges for the 16-mg/kg (male), 16-mg/kg (female)

Table 1. Plasma 4-OHA levels in rats after various oral doses

Time after dose	Dose (mg/kg body weight):			
	Male			Female 16
	32	16	8	
0	ND	ND	ND	ND
0.5	10.9± 6.6	23.8±24.7	2.4±0.6	43±16
1.0	15.1± 7.2	11 ± 4.8	4.4±2.3	64±22
1.5	24.8± 9.6	14.3± 3.9	3.3±1.6	69±33
2.0	19.8±18.5	13.5± 4.8	2.9±0.6	74±26
3.0	17.8±10.9	18.8± 9.5	5 ±1.9	67±16
4.0	21.5±14.9	19.2±11.9	4.8±1.4	73±30
5.0	23.1±17.2	49 ±53	6.2±4.9	69±21
6.0	18.2±11.9	34.7±38.3	5.9±1.3	72±23
7.0	–	23 ±18.5	4.3±1.3	76±14
23.0	5.9± 3.3	–	–	–
24.0	–	13.2±19.3	–	4± 2
25.0	–	–	1.7±1	–
30.0	2.7± 0.9	–	–	–
31.0	–	3.3± 1.9	–	–
32.0	–	–	2.6±2.9	ND

Data represent the mean values (±SD) for 6 animals, expressed as nmol/l. –, not determined; ND, not detectable

Table 2. 4-OHA pharmacokinetics in the rat

Animal dose groups	AUC (mean nmol h l^{-1} ±CV%)	Median AUC (nmol h l^{-1})	C_{max} (mean nmol/l ±CV%)	Median C_{max} (nmol/l)	Median T_{max} (h)s
8 mg/kg	112±50	104	8.6±22	7.1	4.5
16 mg/kg (male)	573±73	490	72.5±72	55.4	3.5
16 mg/kg (female)	1,163±20	1,181	98.0±17	97.1	4.0
32 mg/kg	374±48	366	33.5±53.1	27.1	11.8

C_{max}, peak plasma concentration; T_{max}, time to peak

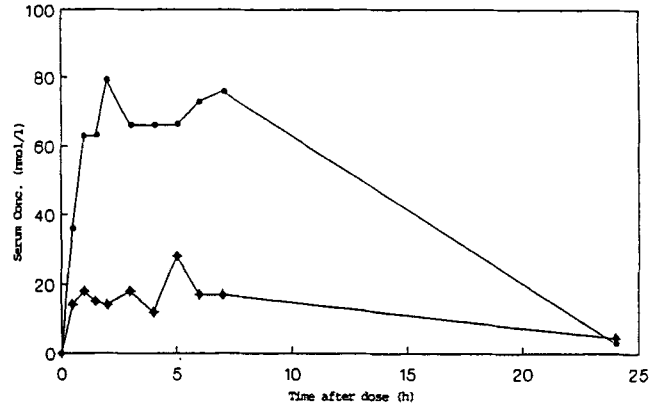

Fig. 1. Median ($n = 6$) serum 4-OHA concentration in male (◆——◆) and female (●——●) rats after a dose of 16 mg/kg

and 32-mg/kg groups were 11.0–49.0, 43.1–76.9 and 10.8–24.9 nmol/l, (0.5–6 h) respectively.

Although the drug levels resulting from a dose of 8 mg/kg were lower than those obtained after the other two doses, overall the 4-OHA concentrations were not proportional to the amount of drug given (Table 1). Of the two groups that received 16 mg/kg, the females tended to show a relatively less marked inter-animal difference in serum 4-OHA values (Table 2); moreover, serum drug concentrations in this group rose more rapidly and persisted at levels higher than those observed in the males. Thus, at 7 h post-dose, 4-OHA values in female rats were between 54.9 and 95.9 nmol/l, whereas in males the corresponding range was 9.6–58.9 nmol/l.

In the male animals the AUC calculated over 0–48 h after the dose was found to show its greatest coefficient of variation in the 16-mg/kg group (AUC, 573 nmol h l^{-1}; CV, 74%). In comparison, the AUC values for the other groups were 112 nmol h l^{-1} ±50% (8-mg/kg group) and 374 nmol h l^{-1} ± 48% (32-mg/kg groups) (Table 2). The AUC (either mean or median values) was not proportional to the amount of drug ingested.

At the same dose level (16 mg/kg), some clear differences according to sex emerged (Fig. 1, Table 2); for example, the mean AUC value for females was more than double that obtained for males, and the inter-animal variations in AUC were lower in females (1,163 nmol h l^{-1} ±20% for females vs 573±74% for males). The median AUC value for the female group (1,181 nmol h l^{-1}; range, 895–1,412 nmol h l^{-1}) was 2.4 times that found for the male group (490 nmol h l^{-1}; range, 274–1,409 nmol h l^{-1}).

After doses of 16 mg/kg, the median C_{max} was 55.4 nmol/l (range, 23.8–115.4 nmol/l), representing the highest obtained in the three dose groups and also the most varied, particularly in the male group (mean value: 98.0 nmol/l ± 17% in females vs 72.5 nmol/l ±72% in males). On the other hand, the median T_{max} values were comparable, with 4 h (range, 1.5–7 h) being noted in females and 3.5 h (range, 0.5–6 h) being observed in males.

Discussion

This study confirms that a sensitive, direct and robust immunoassay is now available for 4-OHA, thus facilitating the incorporation of pharmacokinetic measurements into future clinical studies and trials of this anti-cancer drug.

A feature common to male rats in the present study was that the serum levels of 4-OHA after an oral dose varied most profoundly, irrespective of the dose. Such wide variations, also recently observed in patients [5], precluded the use of the present data for calculation of the standard pharmacokinetic values or for any meaningful comparison between the animal groups. Surprisingly, in female animals this variability was less marked and the bioavailability of the drug was considerably higher than the corresponding values found in males. This might be attributable to differences between the sexes in either the metabolic disposition of 4-OHA or the processes concerned with the intestinal absorption of the drug. We observed that the consistency of the faeces of male rats was invariably loose, whereas the female excreta was normal.

In patients, peak plasma drug levels have been seen 0.5–4 h after ingestion [3–5]. Broadly, a similar pattern was also observed in the present animal study; it appeared as if the drug was absorbed more rapidly from the intestine when the highest dose (32 mg/kg) was given. Of the 4-OHA doses used in the present study, 8 mg/kg most closely corresponds to the p.o. dose of 250 mg that was previously used in the treatment of patients with breast cancer [2–5]. Based on drug concentration/dose, the mean plasma 4-OHA level found in these patients at 1.5 h after the dose was >100-fold that found in rats receiving 8 mg/kg in the present study. The later value, on the other hand, was not vastly different from the 4-OHA levels previously reported in two rats [11].

A plasma half-life of 3 h has been reported for 4-OHA in cancer patients [5]. In subjects given 250 mg oral 4-OHA, the drug was largely undetectable (<2.6 nmol/l) at 24 h after the dose. Our findings suggest that 4-OHA persists longer in the rat and that even by 48 h post-dose, the drug had not been completely removed from the serum. This apparent species difference in circulatory 4-OHA levels after an oral dose could result from the fact that the major route of excretion of this drug appears to be biliary in the rat and urinary in man [6]. Such future studies of 4-OHA should also include the measurement of its glucuronide conjugate to ensure that the role of enterohepatic circulation in 4-OHA pharmacokinetics can be evaluated.

Doubling the dose of 4-OHA from 8 to 16 mg/kg produced proportionally (somewhat) exaggerated rises in serum drug concentrations. In contrast, this response was reversed by a further increase in the dose to 32 mg/kg. This appears to be consistent with the previous finding in cancer patients [7] that the suppression of oestradiol obtained with 250 mg 4-OHA was similar to that achieved with 1,000 mg/day. With this in mind, a possible contribution of the extent of glucuronide conjugation of 4-OHA should be considered [6].

Differences between sexes in the disposition of steroids has been documented [8]. In view of the projected clinical uses of 4-OHA and the present findings in the rat, this aspect of 4-OHA also warrants further investigation. As the current trend in human studies is the use of oral 4-OHA at doses of <250 mg/day [5], it is also important that bioavailability of the drug be determined at different dose levels. The 4-OHA radioimmunoassay used in the present study has the potential of coping with the appropriate analytical demands.

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