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Medroxyprogesterone acetate plasma pharmacokinetics after intravenous administration in rabbits*

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Summary. Medroxyprogesterone acetate (MAP) plasma pharmacokinetics was followed up in a total of 30 New Zealand rabbits after i. v. administration (0.1, 0.5, and 1.0 mg/kg) of either an aqueous suspension or a homogeneous solution of the drug in dimethylsulphoxide (DMSO). A well-defined triphasic decay of MAP plasma levels was noticeable in the animals treated with DMSO solutions. A delayed concentration peak was often present when aqueous suspensions were used, so if is not feasible to fit the experiment with simple polyexponential equations. Model-independent pharmacokinetic analysis (statistical moment theory) revealed a significant dependence of plasma clearance and mean residence time on the dose administered in both conditions.

Introduction

Medroxyprogesterone acetate (MAP) is widely used in the treatment of advanced breast cancer and other hormone-sensitive malignancies. The highest remission rates possible with endocrine therapy in metastatic breast cancer can be achieved with doses of at least 500 mg/day. It has not proved possible to achieve comparable rates with lower doses [5].

Pharmacokinetic studies have recently proved that relatively low drug plasma levels can be obtained by using 'high' MAP doses (2, 6, 7). A comparison of the areas under the time-concentration curves measured after i. p. oral p. o. and i. m. administration [2] showed that a very limited amount of drug is absorbed after p. o administration (0.2%–17.4% of the administered dose, this upper limit computed by assuming complete peritoneal absorption). It also showed that release of MAP from the i. m. injection site is quite slow (7.0–38.0 mg/day for a single 500-mg injection).

Intravenous administration is not considered feasible in humans, because the solubility of MAP in water is so low. This report refers to a pharmacokinetic study carried out on rabbits, in which MAP was administered i. v. both as an aqueous suspension and as a homogenous dimethylsulphoxide (DMSO) solution. The latter is well-known as a potential solvent for water-insoluble drugs.

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Experimental

Animals. A total of 30 New Zealand rabbits of both sexes and weighing 3.5–4.5 kg were used in this study. The animals were kept singly in stainless steel cages at a temperature of 18–20 °C and at a relative atmospheric humidity of 55%–60%. Food (standard Altromin pellets; CL) and water were given freely.

Drug preparation. Commercial vials (Depo-Provera, 150 mg/ml. Upjohn, 1000 mg) were used as MAP source.

The vials were diluted with saline solution to a concentration of 0.9 mg/ml (group WA, rabbits 1–5), 2.25 mg/ml (group WB, rabbits 6–10) and 4.5 mg/ml (group WC, rabbits 11–15).

MAP was also obtained as a pure, crystalline compound by centrifuging the content of the vials and recrystallising the solid residue with acetone. MAP was dissolved in dimethylsulphoxide (Carlo Erba, Milan) at a concentration of 0.8 mg/ml (group DA, rabbits 16–20), 2.0 mg/ml (group DB, rabbits 17–21) and 4.0 mg/ml (group DC, rabbits 18–30).

Drug administration. The drug – both as an aqueous suspension and as a homogeneous DMSO solution – was injected as an i. v. bolus (lasting 30 s) into the marginal vein of the ear.

Rabbits in groups WA and DA were each treated with a dose of 0.1 mg/kg (aqueous suspension and DMSO solution, respectively). The dosage for groups WB and DB was 0.5 mg/kg, while groups WC and DC received 1 mg/kg. Blood samples were obtained from the left ventricle at 3, 5, 10, 20 and 30 min and at 1, 2, 4, 7, 24, 48, 72 and 96 h after administration. Plasma samples were obtained by centrifugation (6000 rpm for 10 min).

Drug analysis. MAP plasma levels were determined by gaschromatography with electron capture detection after extraction and derivatization with heptafluorobutyrric anhydride.

Distilled water (1 ml) and a known amount of internal standard (17-α-hydroxyprogesterone caproate) were added to the plasma samples (1 ml) and extracted twice with cyclohexane (2 × 1 ml) in a vortex mixer for 5 min. After centrifugation, the organic phase was evaporated under vacuum (Buchler Vortex evaporator, 25 mm Hg, 30 °C), and the residue, diluted with acetone/acetonitrile 1 : 1 (500 µl),

was treated with heptafluorobutyric anhydride (Carlo Erba, Milan, 50 µl).

The solution was incubated with stirring for 1 h at 30 °C, and then evaporated under vacuum (25 to 5 mmHg, 30 °C). The residue was reconstituted with 1 ml cyclohexane, and 1-ml fractions were analysed by gas chromatography (Varian model 4600 automated instrument equipped with autosampler model 8000, Ni^{63} Electron Capture detector and Vista 401 data system; 3% SP2250 on 100–120 mesh Supelcoport glass column; injection port temperature 270 °C, detector temperature, 300 °C, column oven program 230 °C to 270 °C, 2 °C/min; carrier gas nitrogen 30 ml/min).

Calibration curves were obtained by spiking blood bank plasma samples with known amounts of MAP (1–1000 ng/ml) and 17-α-hydroxyprogesterone caproate (100 ng/ml). Typical intra- and interassay precision was 5%–8% for MAP plasma levels over 1 ng/ml.

Pharmacokinetic analysis. The MAP concentration time course was simulated whenever possible with a triexponential equation:

$$C = A.\exp[-\alpha.t] + B.\exp[-\beta.t] + C.\exp[-\gamma.t]$$

using the PAR program of the BMDP package [3].

The areas under the time-concentration curve (AUC) and the area under the first moment curve (AUMC)

$$AUC = \int C\,dt$$
$$AUMC = \int t.C\,dt$$

were computed according to the trapezoidal rule and by analytical integration of the polyexponential fit when available. Quite a good relationship was observed between the two computational procedures (see Table 3). Plasma clearance (PlCl), mean residence time (MRT) and volume

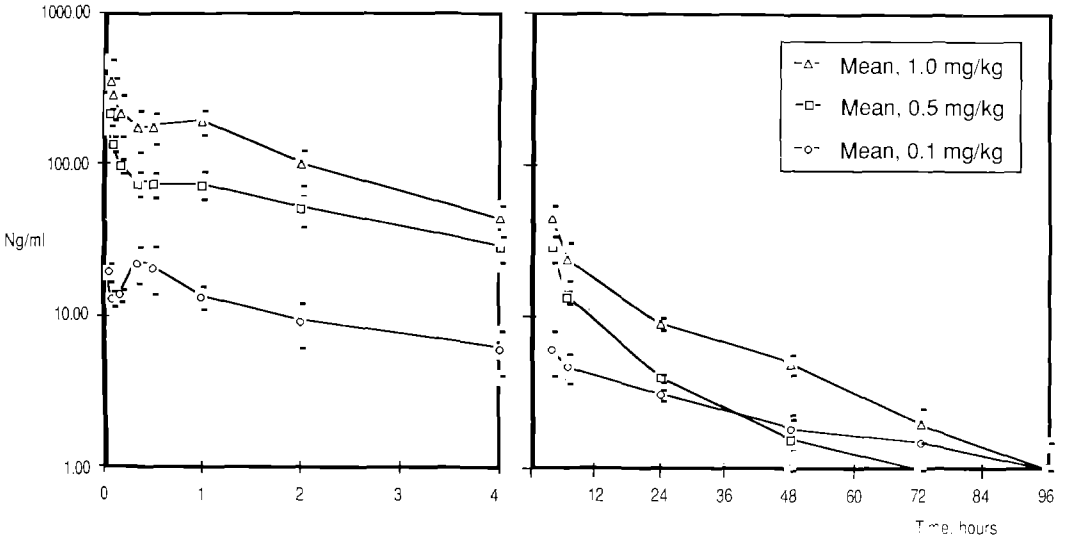

Fig. 1. Medroxyprogesterone acetate (MAP) pharmacokinetics after i. v. administration (aqueous suspension) in rabbits. Mean plasma levels ± SE

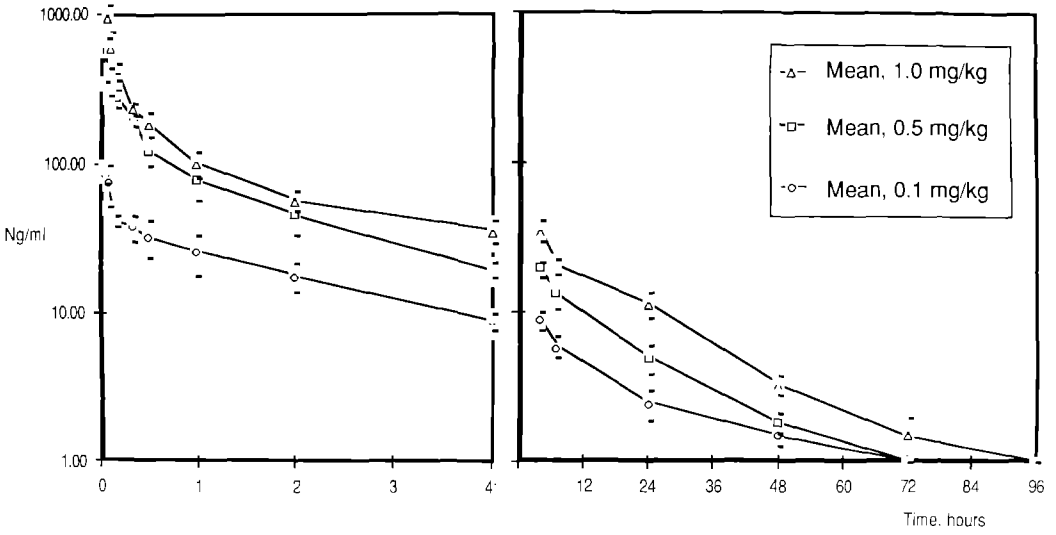

Fig. 2. MAP pharmacokinetics after i. v. administration (DMSO solution) in rabbits. Mean plasma levels ± SE

of distribution at steady state (V_{ss}) were computed according to the statistical moment theory as usual [4]:

$$\begin{aligned} \text{PlCl} &= \text{Dose/AUC} \\ \text{MRT} &= \text{AUMC/AUC} \\ \text{Vss} &= \text{PlCl.MRT} \end{aligned}$$

Results

The animals treated during this study with MAP dosages between 0.1 mg/kg and 1.0 mg/kg – using either an aqueous suspension or a DMSO solution of the drug – survived the experiment and no significant side-effects were recorded.

Table 1. Medroxyprogesterone acetate (MPA) pharmacokinetics after i.v. administration in DMSO solution

	0.1 mg/kg		0.5 mg/kg		1.0 mg/kg	
A	109.4	(SD 28.3)	861.00	(SD 113.1)	1337.1	(SD 303.3)
α	16.2	(SD 4.8)	11.70	(SD 2.5)	18.2	(SD 4.7)
[t/2α]	0.043		0.059		0.038	
B	34.1	(SD 4.1)	218.00	(SD 44.9)	353.7	(SD 65.4)
β	0.63	(SD 0.18)	1.11	(SD 0.30)	1.68	(SD 0.37)
[t/2β]	1.10		0.62		0.42	
C	8.30	(SD 2.4)	23.80	(SD 7.8)	40.5	(SD 8.6)
γ	0.052	(SD 0.01)	0.054	(SD 0.02)	0.056	(SD 0.01)
[t/2γ]	13.33		12.83		12.38	

Coefficients (standard deviations) of the triexponential equation
$C = A.\exp[-\alpha.t] + B.\exp[-\beta.t] + C.\exp[-\gamma.t]$
t/2α,β,γ: Half-lives of the three decay phases (h)

Table 2. Model-independent parameters of MPA pharmacokinetics after i.v. administration in aqueous suspension

Rabbit no.	Dose	AUC	PlCl	MRT	Vss
1	0.1	153.7	2.83	19.3	12.5
2	0.1	308.6	1.28	24.3	7.9
3	0.1	319.5	1.41	23.4	7.3
4	0.1	179.0	2.29	23.6	13.2
5	0.1	178.5	2.18	28.0	15.7
Mean	0.1	227.9	2.00	23.7	11.3
6	0.5	644.6	3.49	6.0	5.3
7	0.5	438.5	4.56	13.6	16.3
8	0.5	444.5	4.28	12.4	12.9
9	0.5	549.4	3.73	20.9	18.9
10	0.5	699.7	2.93	8.9	5.8
Mean	0.5	555.3	3.80	12.4	11.8
11	1.0	883.7	4.19	11.4	12.9
12	1.0	814.6	4.54	11.0	13.5
13	1.0	1155.2	3.29	14.5	12.6
14	1.0	708.5	5.22	13.2	18.7
15	1.0	1964.2	2.14	9.9	5.1
Mean	1.0	1105.2	3.88	12.0	12.6

Dose = administered dose (mg/kg)
AUC, area under time-concentration curve (ng h^{-1} ml^{-1}); PlCl, plasma clearance (l/h); MRT, mean residence time (h); Vss, volume of distribution at steady-state (l/kg)

Table 3. Model-independent parameters of MPA pharmacokinetics after i.v. administration (DMSO solution)

Rabbit no.	Dose	AUC	PlCl	MRT	Vss
16	0.1	182.8	1.97	18.3	10.0
17	0.1	227.6	1.60	16.7	7.4
18	0.1	425.9	0.92	15.3	3.6
19	0.1	234.7	1.58	12.1	5.2
20	0.1	220.2	1.88	18.5	8.4
Mean	0.1	258.2	1.59	16.2	6.9
Exp. int.		222.3	1.71	14.4	6.5
21	0.5	952.5	1.94	10.7	5.6
22	0.5	778.7	2.34	11.0	7.0
23	0.5	841.2	2.11	7.2	4.3
24	0.5	573.1	3.32	13.0	11.4
25	0.5	586.0	3.75	16.9	14.4
Mean	0.5	746.3	2.69	11.8	8.5
Exp. int.		710.5	2.68	11.7	8.3
26	1.0	881.9	4.19	13.6	15.4
27	1.0	831.4	4.21	9.1	11.0
28	1.0	1368.3	2.63	10.7	7.8
29	1.0	837.3	4.60	9.7	11.6
30	1.0	1192.6	3.23	12.5	10.5
Mean	1.0	1022.3	3.77	11.1	11.3
Exp. int.	1.0	1013.1	3.65	13.1	12.9

Exp. int.: Values computed using AUC values determined by integration of triexponential equation

Mean MAP plasma levels are reported in Figs. 1 and 2. The drug plasma concentration time course can be well simulated with a triexponential equation in the rabbits treated with the DMSO solution of MAP. Mean model parameters and standard deviations are reported in Table 1, while model-independent parameters are shown in Tables 2 and 3.

The administration of an aqueous MAP suspension is characterised in several animals by a delayed concentration peak. In this case the drug concentration decay curve cannot be simulated by a simple polyexponential equation. Only the model-independent parameters were computed from the zero and first-order moments of the concentration time course (Tables 2 and 3).

A statistically significant increase in MAP PlCl was observed when the dose administered was increased (Tables 2 and 3; Mann-Whitney rank-sum test; $P=0.0019$ for rabbits treated with aqueous suspension and $P=0.0052$ for DMSO-treated animals). Similarly, drug MRT depended on MAP dosage ($P=0.013$ and $P=0.061$).

Discussion

In spite of its low solubility in plasma, i. v. MAP administration was feasible in rabbits. The use of dimethylsulphoxide (DMSO) to ensure homogeneity of the preparation improved the pharmacokinetic behaviour of the drug, so that the delayed concentration peak observed in the animals treated with an aqueous-suspension was avoided.

The observed increase in MAP plasma clearance and the simultaneous decrease in MRT when a larger dose was administered points to kinetic non-linearity. The liver's

metabolic activity is known to be induced by progestins, and autoinduced MAP elimination is not surprising. It may be interesting to study whether MAP can substantially accelerate the metabolism of other drugs, in view of the high MAP dosages currently used in the management of metastatic breast cancer.

The dosage does not affect the volume of distribution at steady state (Vss), which is indicative of extensive extraplasmatic distribution.

Lastly, the biological MAP half-life ($T^1/_{2\gamma}$) determined in rabbits during this study (12.4–13.3 h) is shorter than the values previously reported in humans (32.1–97.5 h after i. p. [2] and 53.2–64.5 h after p. o [6] treatment). PlCl values determined in this study are lower than those we estimated for humans assuming quantitative drug absorption after i. p. administration, Peritoneal MAP absorption is more favourable than it is after p. o. treatments, although still incomplete.

Further studies are required to determine the actual feasibility of administering MAP i. v. in humans and whether it would be beneficial in the clinical setting. Chronic toxicity and MAP pharmacokinetics after multiple i. v. treatments are currently being investigated in rabbits

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