PLASMA LEVELS OF MEDROXYPROGESTERONE ACETATE (MPA), ESTRADIOL AND PROGESTERONE IN THE RHESUS MONKEY AFTER INTRAMUSCULAR ADMINISTRATION OF DEPO-PROVERA®

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ABSTRACT

Intramuscular injection of medroxyprogesterone acetate (MPA) 150 mg to four mature cycling rhesus monkeys resulted in initially high blood levels of MPA until 3 weeks after injection. Thereafter the concentrations decreased gradually during the remainder of the experiment. MPA concentrations reached below 0.5 ng/ml 70, 83, 90 and 105 days after injection. Ovulatory rises in peripheral plasma levels of progesterone were detected 101, 113, 123 and 233 days after injection, respectively. Despite a 10-fold difference in weight, the same dose of MPA as used in humans (150 mg), resulted in similar peripheral concentrations and duration in the rhesus monkey as in women, as found in a parallel study. The estrogen levels remained within those levels reported for the early follicular phase of the menstrual cycle of untreated monkeys. A significant rise in plasma estrogen levels was found at the end of the experiment. The patterns of plasma MPA, estradiol and progesterone concentrations in the rhesus monkey appear to be related to each other in a similar manner as in women. The metabolism of MPA in rhesus monkey appears to be considerably faster than in women.

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INTRODUCTION

Medroxyprogesterone acetate (MPA) is a widely used contraceptive given 150 mg intramuscularly every 3rd month. Unpredictable bleeding, amenorrhea and delayed return to normal cyclicity after discontinuing have been the major disadvantages of the method (1,2,3,4,5).

The rhesus monkey (Macaca mulatta) is a laboratory animal with a reproductive physiology grossly similar to man in many hormonal aspects (6,7). The usefulness of the rhesus monkey in resolving problems in human contraception is hampered by lack of descriptive data on mode of action in primates of compounds with wide use in women.

This study was undertaken with the aim to correlate plasma levels of MPA with the plasma levels of progesterone and estradiol.

As the rhesus monkeys have been found to have a more rapid metabolism than in man of other progesterone derivatives (8,9), the same dose as in women (150 mg) was chosen, despite the smaller size of the rhesus monkey.

MATERIAL AND METHODS

Four mature rhesus monkeys (Macaca mulatta) with previous regular and ovulatory cycles were chosen. Body weight was 8.6 kg (monkey 99), 5.2 kg (monkey 127), 5.9 kg (monkey 119) and 10.4 kg (monkey 122). The animals were fed and watered ad lib. Each monkey received a single injection of MPA 150 (Depo-Provera®, Upjohn Company) intramuscularly in the gluteal region on day 5 of the menstrual cycle. Rubbing at the site of injection was avoided. Five blood samples were drawn during the first day. The first one a few minutes after injection and the others every two hours for 8 hours. The study continued with daily blood samples for 30 days and later with blood sampling three times per week until the first ovulation was detected through a rise in progesterone levels. Each blood sample was 3 ml and collected from a saphena vein by venipuncture. The blood was placed in heparinized containers, centrifuged and the plasma was frozen until subsequent analyses of MPA, progesterone and estradiol. The monkeys were checked daily for vaginal bleeding.

MPA was measured by radioimmunoassay using an antiserum prepared by Cornette et al. (10). The antiserum was a gift from Dr. Kirton (Upjohn, Kalamazoo) as was the crystalline MPA used as standard. H^3-1,2-MPA with a specific activity of 58 Ci per mM, was purchased from New England Nuclear Corporation and used as tracer in the assay. The serum samples (0.1 - 0.4 ml) were extracted once with petroleum ether (10 volumes) before radioimmunoassay. The sensitivity of the assay was good as 25 pg could clearly be distinguished from zero on the standard curve. The blank of the method was never above 25 pg. No correction was made for extraction losses. One extraction with 10 volumes of petroleum ether recovered 86 per cent of added tritiated MPA. A detailed description of the method is published elsewhere (15).

The plasma levels of progesterone were analysed by a radioimmunoassay

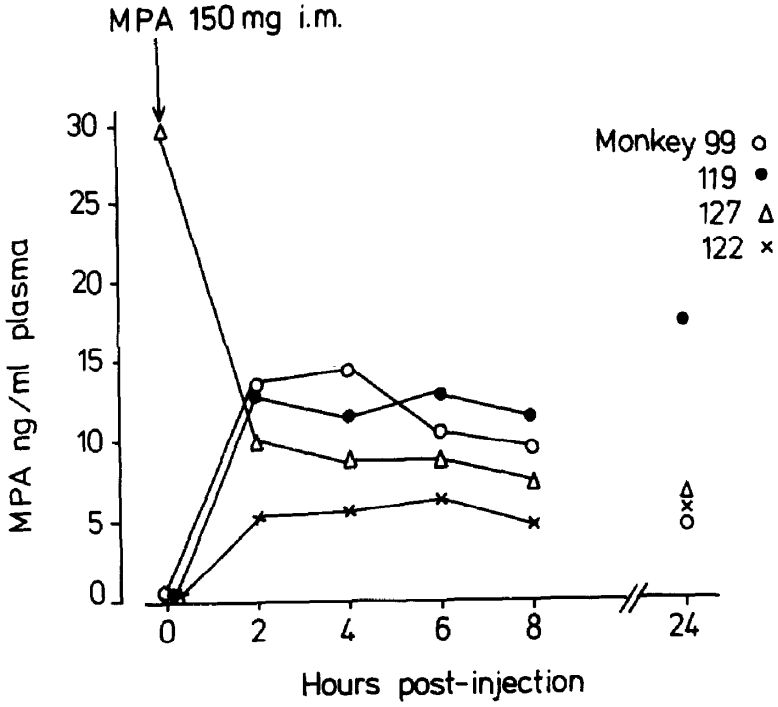

Fig. 1. Serum levels of progesterone, estradiol and MPA during 8 hours following intramuscular injection of 150 mg MPA.

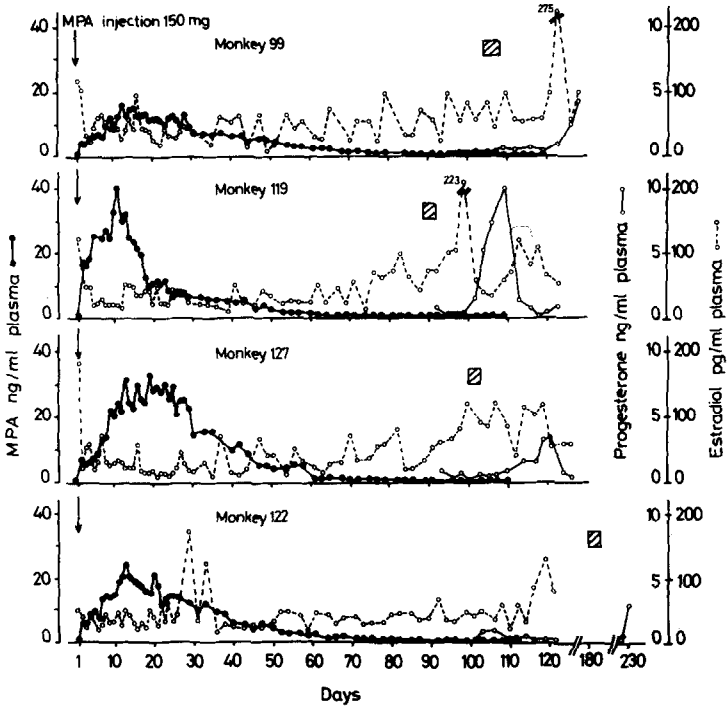

Fig. 2. Serum levels of progesterone, estradiol and MPA following intramuscular injection of 150 mg MPA.

technique (11) with minor modifications (12).

The estradiol levels in plasma were determined by a radioimmunoassay technique (13).

RESULTS

A few minutes following injection the plasma concentrations of MPA were below 0.5 ng/ml in three monkeys and 30 ng/ml in one. Two hours later, MPA concentrations varied between 5 ng/ml to 14 ng/ml and remained in a similar range during the next 8 hours (Fig. 1).

Following the first day, MPA concentrations displayed a rapid rise with the maximum levels during the second and third week after injection. Thereafter MPA concentrations decreased moderately until day 50. The concentrations decreased gradually, with little individual variations, during the remainder of the experiment (Fig. 2). MPA concentrations below 0.5 ng/ml were observed at 70, 83, 90 and 105 days after injection. The first vaginal bleeding was observed at 86, 95, 113 and 174 days after injection. Three monkeys showed the first ovulatory levels of progesterone 30 - 33 days following MPA concentrations below 0.5 ng/ml. One animal(No. 122)continued with low levels of progesterone for 128 days, with MPA levels below 0.5 ng/ml. Ovulation was detected at 101, 113, 123 and 233 days after injection (Fig. 2).

The plasma estradiol levels were related to MPA levels according to three conditions: First, when MPA levels were over 10 ng/ml, second when they were 1 - 4 ng/ml and third when they were below 0.5 ng/ml. The mean plasma estrogen levels were 33.9 pg/ml in the first condition, 41.0 pg/ml in the second and 64.9 pg/ml in the third. Statistical analysis with the t-test showed that the rise in the plasma estrogen levels from the first to the third condition was significant ($p < 0.001$).

DISCUSSION

The principal mode of action of MPA is thought to be inhibition of ovulation (3,4,14,15,16). The return to normal ovulatory cyclicity has been associated with decrease in peripheral drug concentration (2,3). In three of our animals, ovulation inhibition was maintained as long as MPA concentrations were detectable. One monkey (No. 122) persisted with amenorrhea and anovulation despite MPA concentrations below 0.5 ng/ml since day 105. In this animal the end of the experiment coincided with the summer months. The failure to recover normal cyclicity may be due to a summer amenorrhea period (17).

In both humans and rhesus monkeys, 150 mg of MPA given intramuscularly results in similar patterns of peripheral concentrations until the third week after injection but with absolute levels higher in the rhesus monkey. In both species, plasma levels fluctuate initially indicating uneven release from the injection site. The duration of the ovulation block in women after one injection of 150 mg MPA ranges from 90 to 245 days, according to different authors. Cornette et al.(10) and Kirton and Cornette (2) reported that MPA concentrations differ in women by a more

regular and prolonged release after the third week resulting in elevated plasma levels which persist much longer than in monkeys. However, in a parallel study in women (16) using the same method of measurement for MPA as in this study, the MPA levels reached 0.5 ng/ml at a similar time as in the monkeys of this experiment.

Intravenous injection of 100 ug MPA in rhesus monkeys resulted in high concentrations which fell rapidly within 10 minutes after injection (10). One animal (No. 127), showed high levels a few minutes after the injection which probably indicates partial intravenous injection. However, two hours later there was no difference in concentrations as compared with the other animals. This observation shows that MPA once in circulation has a rapid metabolism and removal from the blood stream. Despite the fact that the rhesus monkey is only one tenth of the weight of a woman, 150 mg MPA produced similar concentrations in plasma as in women, indicating a more rapid metabolism of chlormadinone acetate shown in the rhesus monkey (8).

Large clinical studies in women receiving 150 mg MPA every 3rd month have shown unpredictable bleedings mainly at the beginning of therapy (1,2,3,5). The first bleeding in the monkey anticipated a return of ovarian function. At different periods of time after the first vaginal bleeding, all the monkeys recovered ovulation. In women who are amenorrhoeic during treatment, the recovery of vaginal bleedings usually anticipates the recovery of ovulation (1,4,18). However, women can become pregnant during amenorrhea induced by MPA just before the end of an interval injection when the MPA effect has begun to wear off (19).

The estrogen levels in women receiving 150 mg MPA every 3rd month have been reported within the levels found during the early follicular phase of the menstrual cycle in untreated women (16,20). A significant rise in plasma estrogen levels has also been found at the end of the treatment period (16,21). The same pattern in estrogen concentration was found in the rhesus monkeys. At the beginning of therapy, estrogen levels were within the range reported for the normal early follicular phase of rhesus monkey (6,7). Coinciding with MPA concentrations below 0.5 ng/ml, the estrogen levels were significantly higher.

In conclusion the patterns of MPA and ovarian steroids in plasma in the rhesus monkey correlated to each other as in humans. In both species ovulation is blocked at similar plasma levels of MPA. The return to normal ovulatory cyclicity is associated with decreasing plasma levels of the drug. The metabolism of MPA appears to be considerably faster in the rhesus monkey than in women.

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