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Effect of medroxyprogesterone acetate on hepatic and placental drug metabolism in rats

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Medroxyprogesterone acetate (MPA; Depo-Provera) is a long-acting synthetic progestin that has been used as a contraceptive for more than 11 million women-years in 80 nations [1]. The U.S. Food and Drug Administration has denied approval of MPA as a contraceptive because of concern about its safety, especially teratogenicity and carcinogenicity [2, 3]. In addition to its contraceptive action, MPA has been used clinically at high doses for the treatment of breast [4], kidney [5] and prostate cancer [6]. It has also been used for slowing sexual development in children with precocious puberty [7].

A few studies have shown that MPA at high levels induces hepatic drug metabolism in rats [8–10] and humans [11]. Morevoer, MPA increases the rate of steroid metabolism in several species [12, 13]. In contrast, pregnancy is known to decrease hepatic drug metabolism particularly in late pregnancy [14]. Because some women to whom MPA is administered as a contraceptive may be unknowingly pregnant, it is important to know the nature of the combined effects of MPA and pregnancy on drug metabolism. There is no published information on the effect of MPA on either hepatic or placental drug metabolism in pregnant animals. The present study was undertaken to evaluate the combined influences of pregnancy and MPA administration on hepatic and placental drug metabolism in the rat.

Materials and methods

Animals. Female and male Sprague–Dawley rats weighing 175–200 g were purchased from Bantin & Kingman (Fremont, CA). Females were caged individually and males, two per cage, in a climate-controlled facility (21–22°) on a 12-hr light/dark cycle. Food (Purina Rat Chow) and fresh water were available *ad lib.* All animals were allowed to acclimate for 14 days prior to breeding. Successful mating was determined by the presence of sperm in vaginal smears. Day 0 of gestation was established as the day on which sperm was detected. All pregnant animals were monitored for weight gain, gross appearance, and righting reflexes during the course of gestation.

Drug treatment. MPA was obtained from the Upjohn Co. (Kalamazoo, MI) as a microcrystalline suspension in saline. Pregnant rats were administered 0 (saline) 45, 90, 180 or 360 mg/kg. Non-pregnant rats were administered only 0 and 360 mg/kg. A single dose (approximately 0.3 ml) was injected intramuscularly in the right rear flank using a 25 gauge needle on the morning of gestational day 13.

Necropsy and collection of tissue samples. Pregnant and control rats were killed on the morning of gestational day 20 and 7 days post-treatment respectively. The rats were anesthetized by brief exposure to CO_2 and terminated by decapitation. Maternal liver and lungs were inspected for gross evidence of pathologic change. Fetuses were removed and examined for abnormalities; these results are described elsewhere [15]. Livers and placentas were removed quickly, weighed, and chilled in ice-cold 1.15% KCl.

Maternal trunk blood (6–10 ml) for MPA analysis was collected in non-heparinized glass tubes at the time of sacrifice. Serum which was collected from clotted blood by centrifugation at 10° and 1000 *g* for 12 min was stored in sealed glass vials at −20° until quantified for MPA.

Microsomal and cytosolic enzyme assays. Liver or placental tissue was homogenized (1:2, w/v) in 19.8 mM Tris buffer in 1.15% KCl (pH 7.4) using a Polytron (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged at 16,800 *g* for 20 min at 0–4°. The supernatant fracture was then centrifuged at 105,000 *g* for 60 min at 0–4°. The 105,000 *g* supernatant fraction was stored frozen (−20°) for subsequent analysis of cytosolic enzyme activities. The microsomal pellet was resuspended in 4 ml of a solution composed of 50% glycerol, 100 mM Tris buffer and 0.1% BHT and then stored at −20° in sealed vials. Just before use, stored microsomes were combined with 15 ml of a 0.4 M sucrose: 77 mM sodium pyrophosphate solution and centrifuged at 105,000 *g* at 1–4° for 60 min. The microsomal pellet was resuspended in 150 mM KCl and protein concentration was determined [16]. Cytochrome P-450 concentrations were determined according to Omura and Sato [17].

Microsomal activities of aminopyrine *N*-demethylase (APND) and ethoxycoumarin *O*-deethylase (ECOD) were determined as previously described [18]. Substrate concentrations were 40 mM for aminopyrine and 0.80 mM for ethoxycoumarin. Aldrin epoxidase (AE) was measured by a modification of the method of Krieger *et al.* [19], the reaction mixture was the same as in the other monooxygenase assays [18], and the aldrin concentration was 0.55 mM. Aldrin epoxidase assays were terminated by the addition of 5.0 ml *n*-hexane and the immediate cooling of the reaction mixtures in ice. Samples were extracted (Fisher Rotorack) for 20 min, and dieldrin was quantified using a Varian 1200 gas chromatograph equipped with a 3 m glass column packed with OV101 and a tritium foil electron capture detector. All monooxygenase assays were validated for time and protein linearity and were routinely assayed using a microsomal protein concentration of 0.8 mg/ml and a reaction time of 20 min. NADPH cytochrome *c* reductase was measured spectrophotometrically [20] using concentrations of 0.1 mg/microsomal protein assay and 0.257 mM cytochrome *c*.

Microsomal epoxide hydrolase (mEH), cytosolic epoxide hydrolyase (cEH) and glutathione *S*-transferase (GST) were assayed by single-step radiometric partition assay [21]. In brief, microsomes or cytosol were incubated with tritiated substrate (cEH, *trans*-stilbene oxide, pH 7.4; mEH, *cis*-stilbene oxide, pH 9.0; and GST, *cis*-stilbene oxide plus glutathione, pH 7.4) at 37° for 10–30 min, and the epoxide, or epoxide and diol, preferentially extracted with isooctane and hexanol respectively. For cytosolic epoxide hydrolase, diluted cytosols were routinely preincubated with 0.5 mM diethylmaleate for 10 min at room temperature to deplete endogenous glutathione (Moody *et al.*, unpublished data). Cytosolic protein concentration was quantified using the method of Bradford [22] as modified by Moody *et al.* [23].

Analysis of MPA in serum. The radioimmunoassay procedure of Hiroi *et al.* [24] as modified by Prahalada *et al.* [25] was used to quantify MPA in maternal serum samples (5–10 μl).

Statistical analysis. Statistical comparisons between means were calculated using a one-way analysis of variance procedure and the method of Bonferroni [26] to locate differences among means. Linear regression analysis was conducted in which serum MPA concentration or MPA dose (independent variable) and drug metabolism or body weight data (dependent variable) were regressed against each other to determine the degree to which data sets were

correlative with serum MPA concentrations or MPA dose. Data from all sixteen MPA-treated pregnant rats were combined for each calculation.

Results and discussion

The only adverse effect observed over the 7-day period following the single i.m. injection of MPA on day 13 was a significant dose-related decrease in body weight gain of the dams (Table 1). This effect was not the result of a concomitant decrease in either the number or total weight of the fetuses; these results and other measurements on the fetuses were presented in a separate report [15]. Regression analysis indicated that the reduction in maternal weight gain was inversely correlated with serum MPA concentration on day 20 (Table 1) with a correlation coefficient (r) of -0.694. When MPA dose was the independent variable, r was -0.684 indicating that the reduction in maternal body weight gain correlated about equally with either the serum MPA concentration or the dose of MPA. Although we have no definitive explanation for this maternal effect of MPA, one or more hormonal mechanisms are probable. Excessive progesterone is known to decrease growth rate in male rats, but not in females [27]. Perhaps a progesterone-like effect similar to that in males was caused by MPA in late pregnancy. Alternatively, the glucocorticoid-like activity of MPA [28] may have been a factor. However, whereas the inductive behaviour of MPA has been suggested as due to its glucocorticoid-like activity [9], we found no evidence of any association between drug-metabolizing activities and suppression of body weight gain.

Livers were significantly larger in all pregnant rats compared to non-pregnant rats, but MPA had no effect on liver size in either pregnant or non-pregnant rats (Table 2). Similarly, placental weights were not changed by MPA treatment. Also, MPA had no apparent affect on either the components of drug metabolism (i.e. concentrations of cytochrome P-450 and microsomal protein and activity of NADPH cytochrome *c* reductase), or monooxygenases, epoxide hydrolases, and glutathione conjugase activities (Table 3). Others [8–10] have reported induction of hepatic drug-metabolizing enzymes in MPA-treated female rats, but the exposure regimes were quite different from the present study. Saarni *et al.* [9] and Stengard *et al.* [10] observed significant dose-dependent increases in liver size, cytochrome P-450 and b_5 concentrations, and the activities of several hepatic microsomal monooxygenases in rats administered from 10 to 600 mg/kg MPA i.p. daily for 7 consecutive days. Similarly, Jori *et al.* [8] reported significant induction of *p*-nitroanisole, aniline and aminopyrine metabolism in female rats administered 10 mg/kg/day MPA, p.o., for 30 consecutive days. Collectively, these studies showed MPA to be a mixed-type inducer of monooxygenases. Also, NADPH cytochrome *c* reductase was particularly responsive to MPA [9]. The lack of induction in the present study was further confirmed by linear regression analysis which failed to demonstrate any significant correlations between serum MPA concentration and a specific organ or subcellular measurement associated with drug metabolism in either the treated pregnant or non-pregnant rats.

Table 2. Effect of MPA on liver and placenta weights in pregnant rats and liver weights in non-pregnant rats

Dose of MPA (mg/kg)	Liver (g)		Placenta (g)
	Pregnant	Non-pregnant	
0	15.4 ± 2.1*	11.8 ± 1.1†	11.1 ± 0.8
45	16.3 ± 0.9	ND‡	10.5 ± 1.6
90	16.6 ± 1.6	ND	10.2 ± 1.1
180	14.7 ± 2.0	ND	9.5 ± 1.1
360	15.6 ± 1.1	12.7 ± 0.9†	9.0 ± 0.5

* Mean (± S.D.) of four measurements per treatment.
† Significantly different from liver weights of pregnant rats, $P < 0.05$.
‡ ND = not determined.

All of the microsomal enzymes (i.e. APND, AE, ECOD, and EH) except NADPH cytochrome *c* reductase were significantly less active in pregnant rats (Table 3). Conversely, neither of the cytosolic enzymes measured (i.e. EH and GST) was affected by pregnancy. These results confirm the reports of others that hepatic microsomal drug-metabolizing activities are decreased in late pregnancy [14, 29, 30], and that this decrease is not accompanied by concomitant decreases in either cytochrome P-450 concentration or NADPH cytochrome *c* reductase activity [29]. Moreover, the difference in the effect of pregnancy on mEH versus cEH is consistent with the suggestion that microsomal and cytosolic EH enzymes are under independent regulation [31]. However, the lack of an effect on GST differs from the results of Polidoro *et al.* [32] who reported a 70% activity increase in late pregnancy using 1,2-epoxy-3-(*p*-nitrophenoxy) propane as a substrate, but this pregnancy effect was substrate-specific.

Neale and Parke [14] reported that inducers such as phenobarbital and methylcholanthrene nullify the inhibitory effect of pregnancy on drug-metabolizing enzymes. Because MPA was unresponsive in the present study, this phenomenon could not be tested. Whether MPA is capable of having a nullifying effect under experimental conditions that produce a definitive induction response (e.g. greater dosage administered p.o.) remains to be studied.

There were no significant dose-dependent effects of MPA on the activities of microsomal monooxygenases, mEH, cEH, or GST in the placenta (Table 4). Both cytochrome

Table 1. Maternal body weight gain during gestation, both before and after MPA treatment, and concentrations of MPA in serum collected on day 20

MPA dose (mg/kg)	Body weight gain (g)		MPA in serum (ng/ml)
	Before MPA, days 0–13	After MPA, days 13–20	
0	70.0 ± 7.2*	90.5 ± 9.7	<0.6†
45	59.8 ± 3.8	89.5 ± 10.4	17.9 ± 16.4
90	62.5 ± 3.0	67.8 ± 16.4	45.5 ± 19.2
180	63.5 ± 6.0	66.5 ± 14.2	45.6 ± 32.8
360	63.8 ± 9.9	55.5 ± 15.0†	143.8 ± 48.3

* Mean (± S.D.) of four measurements per treatment group.
† Minimum detectable level.
‡ Significantly different from control and 45 mg/kg values, $P < 0.05$.

Table 3. Effects of increasing doses of MPA on hepatic drug metabolism in pregnant and non-pregnant rats

Dose of MPA (mg/kg body wt)	Microsomal protein*	Cytochrome P-450†	NADPH cytochrome *c* reductase‡	Aminopyrine *N*-demethylase§	Aldrin epoxidase§	Ethoxycoumarin *O*-deethylase§	Epoxide hydrolase§ (microsomal)	Epoxide hydrolase‖ (cytosolic)	Glutathione *S*-transferase‖
Pregnant rats									
0	12.49 ± 1.68¶	0.24 ± 0.18	175 ± 10	4.48 ± 0.70	0.18 ± 0.02	0.33 ± 0.06	7.70 ± 2.93	0.16 ± 0.08	19.4 ± 13.7
45	13.47 ± 0.96	0.28 ± 0.06	166 ± 28	4.81 ± 0.30	0.25 ± 0.02	0.32 ± 0.08	7.65 ± 1.95	0.14 ± 0.03	17.9 ± 8.9
90	12.27 ± 1.12	0.23 ± 0.12	164 ± 40	5.71 ± 0.46	0.38 ± 0.28	0.33 ± 0.06	6.65 ± 0.71	0.16 ± 0.07	20.6 ± 11.2
180	12.22 ± 3.98	0.21 ± 0.04	175 ± 36	5.15 ± 0.32	0.31 ± 0.29	0.33 ± 0.06	6.25 ± 1.28	0.14 ± 0.04	12.3 ± 5.4
360	13.82 ± 2.92	0.23 ± 0.06	172 ± 34	5.74 ± 0.60	0.25 ± 0.10	0.33 ± 0.02	7.08 ± 3.06	0.12 ± 0.03	11.3 ± 4.4
Non-pregnant rats									
0	11.63 ± 1.48	0.34 ± 0.16	158 ± 12	7.88 ± 1.64**	1.45 ± 1.05**	0.53 ± 0.22**	14.62 ± 2.00**	0.17 ± 0.07	19.9 ± 18.4
360	10.58 ± 0.96	0.29 ± 0.06	160 ± 12	7.46 ± 2.60**	1.58 ± 0.57**	0.49 ± 0.20**	17.35 ± 6.15**	0.18 ± 0.07	24.2 ± 14.6

* Units are mg microsomal protein/g of liver (wet wt).
† Units are nmoles/mg microsomal protein.
‡ Units are nmoles of cytochrome *c* reduced/min/mg microsomal protein of product formed.
§ Units are nmoles/min/mg microsomal protein.
‖ Units are nmoles/min/mg cytosolic protein.
¶ Mean (± S.D.) of data from four animals.
** Designates mean that is significantly different ($P < 0.05$) from other means in the same row.

Table 4. Effect of increasing doses of MPA on placental drug metabolism

Dose of MPA (mg/kg body wt)	Microsomal protein*	Cytochrome P-450	NADPH cytochrome *c* reductase†	Aminopyrine *N*-demethylase‡	Aldrin epoxidase	Ethoxycoumarin *O*-deethylase‡	Epoxide hydrolase§ (microsomal)	Epoxide hydrolase§ (cytosolic)	Glutathione *S*-transferase‖
0	1.96 ± 0.29¶	ND**	73.9 ± 3.4	1.03 ± 0.34	ND	0.13 ± 0.03	99.4 ± 39.7	14.20 ± 1.90	59.5 ± 3.5
45	2.18 ± 0.08	ND	78.5 ± 1.3	0.83 ± 0.44	ND	0.14 ± 0.03	80.8 ± 21.8††	10.50 ± 1.15	57.9 ± 15.6
90	1.84 ± 0.22	ND	77.2 ± 10.6	0.56 ± 0.10	ND	0.12 ± 0.04	231.8 ± 83.1††‡‡	10.65 ± 0.73	48.4 ± 9.2
180	2.36 ± 0.18	ND	80.5 ± 12.0	0.78 ± 0.16	ND	0.13 ± 0.04	80.9 ± 56.4	9.68 ± 1.86	51.4 ± 7.1
360	2.51 ± 0.32	ND	74.5 ± 4.4	0.74 ± 0.22	ND	0.13 ± 0.04	70.8 ± 14.5††	8.13 ± 1.93	45.1 ± 16.3†

* Units are mg microsomal protein/g of placenta (wet wt).
† Units are nmoles of cytochrome *c* reduced/min/mg microsomal protein.
‡ Units are nmoles of product formed/min/mg microsomal protein.
§ Units are pmoles of product formed/min/mg microsomal protein.
‖ Units are pmoles of product formed/min/mg cytosolic protein.
¶ Mean (± S.D.) of data from four animals except where indicated.
** ND = not detectable.
†† N = 3.
‡‡ Designates mean that is significantly different ($P < 0.05$) from other means in the same row.

P-450 concentration and AE activity were too low to measure. The only significant response to MPA was a greater than 2-fold stimulation of mEH at the 90 mg/kg dose level, but we have no explanation for this specific reponse. Linear regression analysis revealed no significant correlations between MPA concentrations in serum and any placental drug metabolism parameter.

In summary, a single i.m. injection of MPA caused a marked dose-dependent reduction in maternal body weight gain of rats in late pregnancy over a 7-day experimental period, but this response was not accompanied by any changes in hepatic or placental drug-metabolizing activity. MPA was administered in a manner similar to human contraceptive use, i.e. a single i.m. injection of a commercial water-soluble preparation. The experimental dosages of 45, 90, 180 and 360 mg/kg body weight corresponded approximately to 15, 30, 60, and 120 times the Human Contraceptive Equivalent (HCE). The results confirmed that the activities of some hepatic microsomal monooxygenases are decreased significantly during late pregnancy, whereas cytosolic enzymes such as EH and GST are not affected by pregnancy. Moreover, MPA showed no evidence of counteracting the inhibitory effects of pregnancy on hepatic drug metabolism.

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REFERENCES

1. P. C. Schwallie, in *Long-acting Contraceptive Delivery Systems* (Eds. G. I. Zatuchi, A. Goldsmith, J. D. Shelton and J. J. Sciarra), p. 566, Harper & Row, Philadelphia, PA (1984).
2. A. Rosenfield, D. Maine, R. Rochat, J. Shelton and R. A. Hatcher, *J. Am. med. Asso.* **249**, 2922 (1983).
3. M. Sun, *Science* **226**, 950 (1984).
4. F. Pannuti, A. Martoni, A. R. DiMarco, E. Piana, F. Saccani, G. Becchi, G. Mattioli, F. Barbanti, G. Marra, W. Persiani, L. Cassiari, F. Spagnolo, D. Palenzona and G. Rocchetta, *Eur. J. Cancer* **15**, 593 (1979).
5. H. J. G. Bloom, *Br. J. Cancer* **15**, 593 (1979).
6. S. Rafla and R. Johnson, *Curr. Ther. Res.* **16**, 261 (1974).
7. H. S. Kupperman and J. A. Epstein, *J. clin. Endocr. Metab.* **22**, 456 (1962).
8. A. Jori, A. Bianchetti and P. E. Prestini, *Eur. J. Pharmac.* **7**, 196 (1969).
9. H. Saarni, J. T. Ahokas, N. T. Karki, O. Pelkonen and E. A. Sotaniemi, *Biochem. Pharmac.* **29**, 1155 (1980).
10. J. H. Stengard, H. Saarni and E. A. Sotaniemi, *Pharmacology* **28**, 34 (1984).
11. E. A. Sotaniemi, T. Hynynen, J. Ahlquist, J. T. Ahokas, U. Puoskari and I. Pelkonen, *J. Med.* **9**, 117 (1978).
12. G. G. Gordon, A. L. Southren, S. Tochimoto, J. Olivo, K. Altman, J. Rand and L. Lemberger, *J. clin. Endocr. Metab.* **30**, 449 (1970).
13. G. G. Gordon, K. Altman, A. L. Southren and J. Olivo, *J. clin. Endocr. Metab.* **32**, 457 (1971).
14. M. G. Neale and D. V. Parke, *Biochem. Pharmac.* **22**, 1451 (1973).
15. E. G. Carroad, S. Prahalada and A. G. Hendrickx, *Tetratology* **31**, 51A (1985).
16. A. C. Gornall, A. J. Bardwill and M. M. David, *J. biol. Chem.* **177**, 751 (1949).
17. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
18. L. R. Shull, M. R. Bleavins, B. A. Olson and R. J. Aulerich, *Arch. environ. Contam. Toxic.* **11**, 313 (1982).
19. R. I. Krieger, S. I. Gee, J. L. Miller and T. Thongsinthusak, *Drug Metab. Dispos.* **4**, 28 (1976).
20. T. C. Pederson, and J. A. Buege and S. D. Aust, *J. biol. Chem.* **248**, 7134 (1973).
21. S. S. Gill, K. Ota and B. D. Hammock, *Analyt. Biochem.* **131**, 273 (1983).
22. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
23. D. E. Moody, D. N. Loury and B. D. Hammock, *Toxic. appl. pharmac.* **78**, 351 (1985).
24. M. Hiroi, F. Z. Stanczyk, U. Gobelsmann, P. F. Brenner, M. E. Lumkin and D. R. Mishell, *Steroids* **26**, 373 (1975).
25. S. Prahalada, E. Carroad, M. Cukierski and A. G. Hendrickx, *Teratology* **32**, 421 (1985).
26. J. Gill, *Design and Analysis of Experiments in the Animal and Medical Sciences*, Vol. 1, p. 177. Iowa State University Press, Ames, IA (1978).
27. A. E. Fahim and D. G. Hall, *Am. J. Obstet. Gynec.* **106**, 183 (1970).
28. E. M. Glenn and S. W. Bowman, *Metabolism* **8**, 265 (1959).
29. B. Testa and P. Jenner, *Drug Metabolism: Chemical and Biochemical Aspects*, p. 410. Marcel Dekker, New York (1976).
30. G. Feuer, *Drug Metab. Rev.* **9**, 147 (1979).
31. R. N. Wixtrom and B. D. Hammock, in *Biochemical Pharmacology and Toxicology* (Eds. D. Zakim and D. A. Vessey), Vol. 1, p. 1. John Wiley, New York (1985).
32. G. Polidoro, C. Dillio, A. Arduini and G. Federici, *Biochem. Pharmac.* **30**, 1859 (1981).

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