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ORIGINALS

Radioimmunoassays of Ethinyl-Norgestrienone (R–2323) and Medroxyprogesterone Acetate (MPA) and Their Clinical Applicability

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Summary. Radioimmunoassays for 2 synthetic progestins (Ethinyl-norgestrienone, R 2323 and medroxyprogesterone acetate, MPA) are demonstrated. 10 patients aged 31 to 72 years were treated with ethinyl-norgestrienone with different schedules and 3 men suffering from benign prostatic hypertrophy were treated with medroxyprogesterone acetate. Plasma levels of testosterone, LH, FSH were monitored before, during and after treatment.

Key words: Ethinyl-norgestrienone (R-2323) - Medroxyprogesterone acetate (MPA) - Radioimmunoassay.

Ethinyl-norgestrienone (17-α-ethinyl-17-hydroxy-18-methyl-4, 9, 11-estrien-3-one, R 2323) and medroxyprogesterone acetate (16-methyl, 17-α-hydroxyprogesterone-acetate, MPA) are synthetic progestins which have been in clinical use for some years. Both compounds have proved to be potent inhibitors of ovarian function in the female, and of spermatogenesis and accessory gland function in the male. The aim of the present study was to establish methods for determining the concentrations of R-2323[1] and MPA[2] in human plasma, in order to acquire more information about their mode(s) of action.

SUBJECTS AND REGIMENS

Two male patients, aged 60 and 62 years, were given 12.5 mg R-2323 orally at 6 p.m. daily for 10 days. Blood was drawn at 8 a.m. on each subsequent day, and assayed for plasma R-2323 and testosterone (T) levels.

7 patients aged 61-72 years, suffering from benign prostatic hypertrophy, were treated with 100 mg R-2323 weekly for 6 weeks. Blood was taken at regular intervals to determine plasma levels of T, LH, FSH, and R-2323.

A 31-year-old patient with haematospermia was given 100 mg R-2323 orally per week for 6 weeks, and 50 mg weekly for another 6 weeks. During the treatment period, blood was regularly drawn for determination of plasma T, LH, FSH, and R-2323 levels. The effect on spermatogenesis was studied by regular spermiograms.

The last group consisted of 3 patients with benign prostatic hypertrophy, aged 77, 71 and 58 years. The 77 year old man received MPA in a single intramuscular injection of 1000 mg, the 71 year old patient 500 mg and the patient aged 58 250 mg. Prior to the injection, and on every second day for 16 days thereafter, blood was taken to determine plasma levels of MPA and T.

ASSAY PROCEDURES

R-2323 levels were determined according to the method of Viinikka and his coworkers (11). The dilution of the antiserum[3] of 1 : 3.000 was made

[1] R-2323 tablets were a gift from the Roussel Company, Romainville, France.

[2] MPA was purchased from Farmitalia, Milan, Italy, and from the Upjohn Co., Kalamazoo, Michigan, U.S.A.

[3] The R-2323 antibody and radioactive steroid were kindly given by Dr. J.P. Raynaud, Roussel UCLAF, Romainville, France.

with 0.1% gelatin in phosphate-buffered saline (PBS), pH 7,0. The radioactive steroid (specific activity 45 curie/mmol) was tritiated at positions 6 and 7. The dilution, containing about 8000 cpm/100 µl, was made in PBS with 0.1% gelatin. All reagents used were of analytical purity, and redistillation was not necessary.

1 ml of plasma was extracted with 5 ml of a 1 : 1 mixture of diethyl ether/ethyl acetate, and the extract was evaporated to dryness. About 8000 cpm of radioactive steroid and 100 µl of the chosen antibody dilution were incubated with the test samples for 30 minutes at room temperature and then overnight at 4°.

The bound and unbound fractions were separated with the aid of dextran-coated charcoal. The radioactivity was counted using a Nuclear-Chicago Liquid Scintillation Counter. 10 ml of modified Bray's solution were added to the counting vials. The concentration of R-2323 in the test samples was calculated from standard curves based on standard samples run in parallel with the test samples.

Figure 1 shows a typical standard curve obtained from the means of nine samples at each concentration. The accuracy, reproducibility and specificity of the method are excellent. The plasma controls approximate to 0.1 ng/ml.

Plasma testosterone determinations were done by radioimmunoassay according to the method of Bartke and coworkers (1)[4]. The mean plasma testosterone level in young healthy men is 0.52 ± 0.11 µg/100 ml plasma.

LH and FSH levels were determined by means of the modified solid-phase radioimmunoassay method of Crosignani and coworkers (4).[5]

The range for normal LH values lies between 9 and 13 mU/ml, and for FSH between 10 and 19 mU/ml.

MPA determination was done by the method of Edqvist and Johansson (5). The antiserum[6] was diluted 1 : 10,000.[7] The plasma samples (0.1 to 0.5 ml as needed) were extracted with diethyl ether. The plasma blanks were about 25 pg/ml. The assay has high specificity and reproducibility.

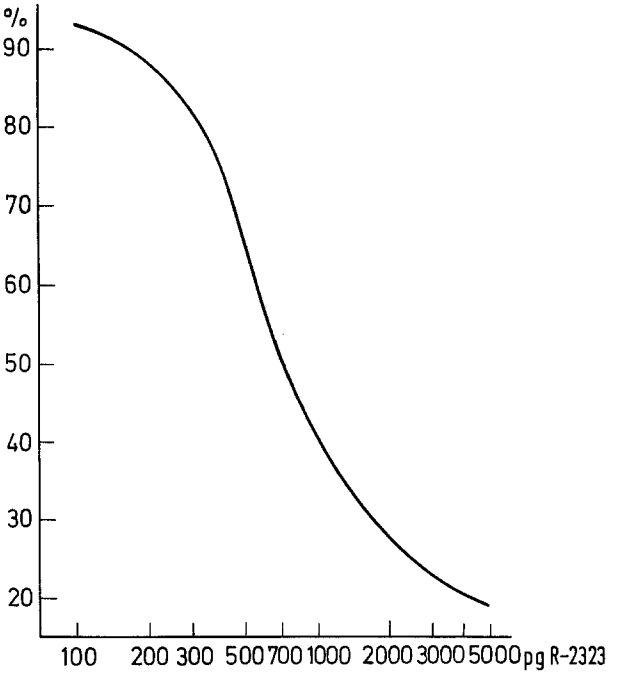

Fig. 1. A typical standard curve obtained from the means of nine analyses. Between 100 and 5000 pg is a 70% decrease of bound radioactivity

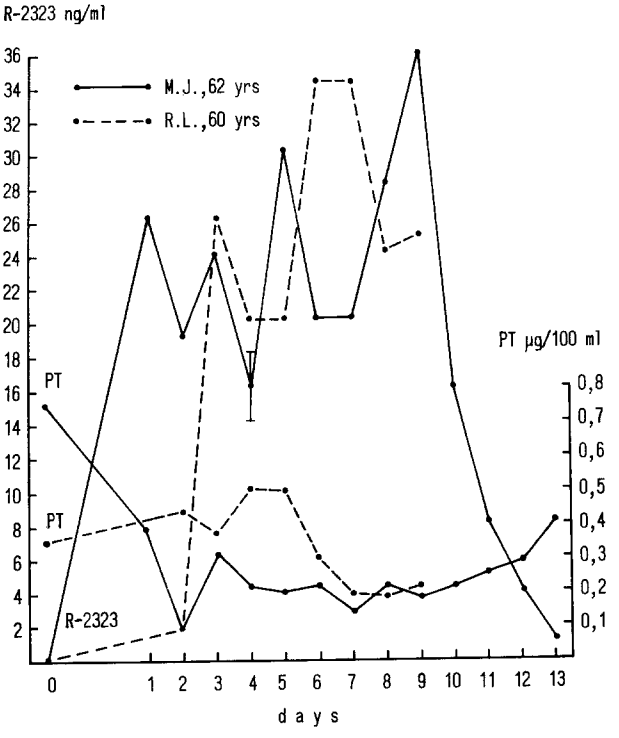

Fig. 2. Plasma R-2323 and testosterone levels of two elderly men who took 12.5 mg R-2323 daily for 10 days

[4]The antiserum was a gift from Dr. K. Sundaram of the Population Council, New York.

[5]The immunological material, LER 907 and second IRP standard, was obtained from the NIH, Bethesda, Maryland.

[6]The antiserum was a gift from Dr. K. T. Kirton, Upjohn Co. Kalamazoo, Michigan, USA. (9, 10)

[7]Tritiated MPA with a specific activity of 58 curies/mmol was purchased from New England Nuclear.

Table 1. Effect of 100 mg R-2323/week orally for 6 weeks on plasma LH, FSH and testosterone levels. The group consists of 7 men aged 61 to 72 suffering from BPH

Pat.	Age	Plasma testosterone (µg/100 ml)			Plasma LH (mU/ml)			Plasma FSH (mU/ml)		
No.		pre	6 wks	12 wks	pre	6 wks	12 wks	pre	6 wks	12 wks
1	64	0.40	0.06	0.07	14.5	7.7	4.4	20.0	10.3	28.0
2	64	0.26	0.08	0.75	14.7	7.9	30.0	33.0	15.0	33.0
3	62	0.65	0.04	0.60	15.0	3.8	10.9	28.0	14.0	30.0
4	69	0.33	0.07	0.38	26.5	11.0	14.0	33.0	16.0	28.0
5	61	0.25	0.05	0.42	5.0	3.6	7.7	19.0	11.0	28.0
6	72	0.54	0.05	0.42	3.5	5.1	10.3	32.0	16.0	30.0
7	63	0.44	0.03	0.04	23.0	5.0	7.1	22.0	16.0	28.0

RESULTS

Individual R-2323 values showed a relatively wide range, from 16 to 36 ng/ml. A sharp rise in plasma R-2323 concentration appeared within 48 hours of the first oral dose. A decrease in plasma T levels, indicating inhibition of Leydig cell function, took place within 2 to 6 days of starting treatment (Fig. 2).

As seen in Table 1, treatment with 100 mg R-2323 weekly for 6 weeks was accompanied by a drastic decrease in plasma T levels in all subjects. Similar changes were found for LH and FSH. In spite of low plasma T levels, these patients had more or less normal sexual activity. The only change in libido occurred during the first 2 to 3 weeks of treatment; thereafter, sexual activity was normal again. Six weeks after treatment was stopped, hormone levels had returned to within the normal range in 80% of the subjects. The R-2323 plasma levels of these patients (Fig. 3) showed great variation throughout the treatment period. Nevertheless, it seems that relatively low R-2323 levels may produce pituitary inhibition.

LH, FSH and T plasma levels of the 31-year-old man treated with R-2323 decreased significantly during the treatment period (Fig. 4). From the sixth to the thirteenth weeks, the plasma T levels were less than 1 ng/ml (B). Complete inhibition of spermatogenesis occurred by the end of six weeks (D), and azoospermia was then maintained for 7 weeks. During that time, the plasma R-2323 level did not exceed 12 ng/ml (C). After treatment was stopped at the twelfth week, the hormone levels returned to normal within a few weeks. Spermatogenesis was normal 3 months later.

Two days after the injection of either 1000 mg, 500 mg or 250 mg MPA, plasma levels of MPA were between 3 and 14 ng/ml (Fig. 5). The plasma T content decreased in each of these 3 patients simultaneously from age specific normal levels to around 1 ng/ml and remained at this level for 4 weeks after the injection.

There was a relatively large variation in individual plasma MPA values between the 3 different administration schedules during the first 18 days following injection. Even after four weeks, the values ranged between 6 and 10 ng/ml.

DISCUSSION

During the past two years, MPA and R-2323 have been used for treatment of various urological disorders: in so-called congestive prostatovesiculitis; in haematospermia (usually, due to hyperplasia of the epithelium of the seminal vesicles); as conservative therapy for prostatic hyperplasia (BPH); and at very high doses of 1000 mg/4-6 weeks, in selected patients suffering from carcinoma of the prostate or kidney.

It will be extremely advantageous to have sensitive methods available for the measurement of plasma levels of administered synthetic steroids. They are already providing much more detailed knowledge of the mode of action of these hormones, the dosage required and the prolonged effects of a depot injection (12). They will also provide the means for monitoring a particular individual's response to treatment. In the study reported here, after an injection of 250, 500 or 1000 mg MPA, there was much less difference in plasma MPA levels than had been expected. This suggests that MPA is stored in

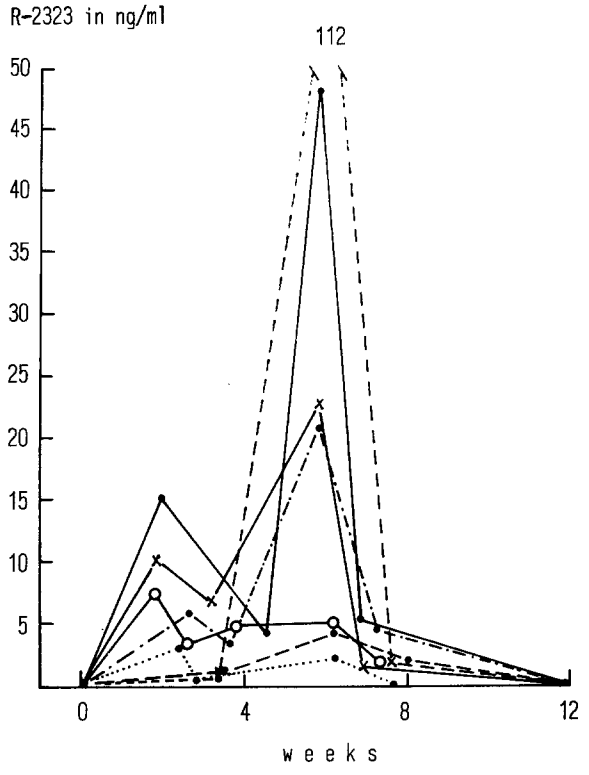

Fig. 3. Plasma levels of R-2323 in ng/ml of the same seven patients as in Table 1, treated with 100 mg R-2323/week orally for 6 weeks

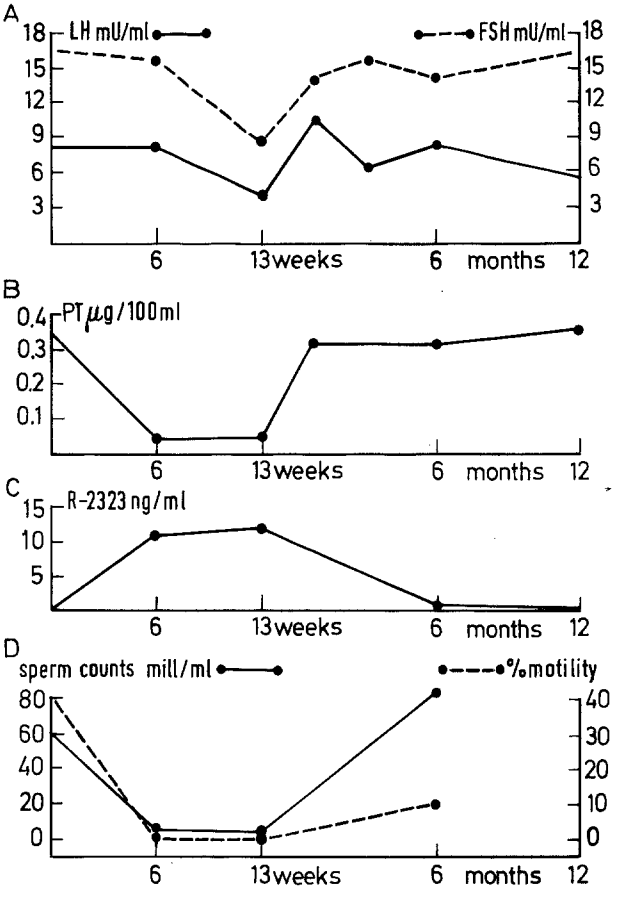

Fig. 4. Plasma LH, FSH, testosterone, R-2323 levels, sperm counts and sperm motility before and after treatment in a 31 year old man suffering from haematospermia who was treated with 100 mg R-2323/week orally for six weeks and 50 mg weekly for six weeks

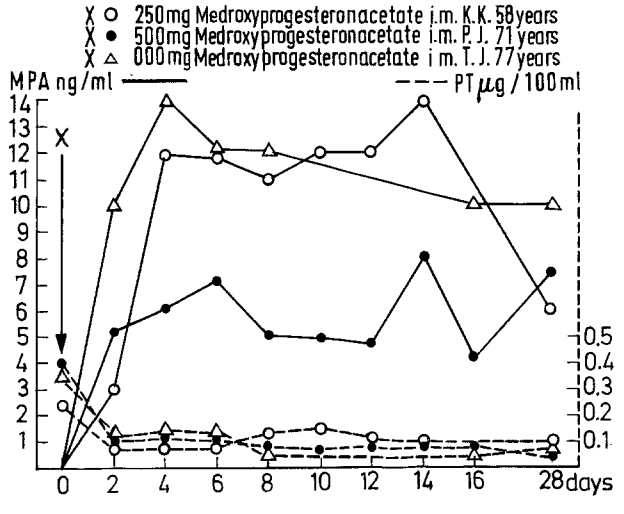

Fig. 5. Data on MPA plasma levels in ng/ml and on plasma testosterone levels of 3 patients suffering from BPH aged 77, 71 and 58. Each of the subjects had a different administration schedule of MPA

body compartments such as fat. In all the patients treated with R-2323, there was a drastic decrease in plasma T levels and in plasma LH and FSH. Even when the dose of R-2323 was halved, 6 weeks after treatment started, these levels remained low suggesting that this regimen would still provide a Leydig cell and sperm suppressing dosage (Fig. 4, B and D).

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