Pharmacokinetics and pharmacodynamics of 7α-methyl-19-nortestosterone after intramuscular administration in healthy men

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7α-Methyl-19-nortestosterone (MENT) is a potent synthetic androgen that is resistant to 5α-reductases and therefore less prone to over-stimulate the prostate. It is a good candidate for implant administration in long-term androgen replacement therapy for hypogonadal men or as part of a male contraceptive system. To investigate the pharmacokinetics of MENT after i.m. administration, single i.m. injections of 2, 4 or 8 mg of micronized MENT were given in aqueous suspension to 18 healthy men in two clinics. Blood was sampled frequently for 8 h and 1, 2, 3, 4 and 9 days after the injections. Serum MENT concentrations were determined by radioimmunoassay. Peak MENT concentrations were dose-dependent and were reached about 1–2 h after the injections. Doubling the dose of MENT resulted in an increase of 60% in peak serum MENT concentrations. The mean ± SE clearance rate was 1790 ± 140 l/day. The antigonadotrophic activity of MENT was investigated by giving six consecutive daily i.m. injections of 1, 2 or 4 mg of MENT to 24 healthy men in two clinics. Blood was sampled before each injection and up to 24 days after the last injection. Serum testosterone and gonadotrophin concentrations (determined by radioimmunoassay and fluoroimmunoassay respectively) decreased in a dose-dependent and statistically significant manner. The highest dose caused a 74% fall in testosterone, a 70% fall in luteinizing hormone, and a 57% fall in follicle stimulating hormone concentrations. MENT injections did not cause any side-effects. The results show that MENT is a potent antigonadotrophic agent in men.
Key words: androgens/male contraception/7α-methyl-19-nortestosterone/pharmacokinetics

Introduction

7α-Methyl-19-nortestosterone (MENT) is a synthetic androgen whose biological potency in animals is several-fold higher than that of testosterone. Compared with testosterone, the relative potency of MENT on muscle and the pituitary is 10–12-fold higher, while its potency on the prostate and seminal vesicles is only 4–5-fold higher. This difference in relative potency is due to the fact that MENT does not undergo 5α-reduction (Agarwal and Monder, 1988) so its actions on the prostate are not amplified, as are those of testosterone (Kumar *et al.*, 1992). Hence MENT is less likely to over-stimulate the prostate when administered at doses that will maintain normal male sexual characteristics. The high potency of MENT makes it suitable for sustained release administration. Hence, MENT is a good candidate to be used as the androgenic component of a hormonal male contraceptive and in long-term androgen replacement therapy of hypogonadal men (Sundaram *et al.*, 1993).

The pharmacokinetics and acute pharmacodynamic changes after i.m. administration of MENT have not been studied previously in men. Although the administration of MENT in the future will not be i.m. (Sundaram *et al.*, 1993), information on the basic pharmacokinetic and pharmacodynamic properties of MENT is necessary for the development of MENT dosage forms suitable for long-term androgen replacement. We therefore measured the concentrations of MENT, testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) before and after single i.m. MENT injections, as well as during and after a series of six i.m. MENT injections given at 24 h intervals.

Materials and methods

Subjects

The studies were conducted in the Family Planning Clinic of The Family Federation of Finland (Väestöliitto), Helsinki, Finland and in the Consultorio de Planificación Familiar, Instituto Chileno de Medicina Reproductiva, Santiago, Chile. In both clinics the same numbers of subjects were studied in each of the protocols. In study 1 the effects of a single i.m. injection were determined in 18 healthy men. The nine Finnish subjects ranged in age from 22 to 39 years (mean ± SE, 32 ± 1.9). Their mean body weight was 80 ± 2.5 kg (range 71–95), and body mass index 24.6 ± 0.7 kg/m² (range 21.4–27.8). The nine Chilean subjects ranged in age from 19 to 36 years (26 ± 1.6). Their mean body weight was 67 ± 2.5 kg (range 55–81), and body mass index 23.6 ± 1.1 (range 19.0–28.5). Three subjects in each clinic were slightly overweight (body mass index 25–30).

In study 2 the effect of six consecutive daily injections of MENT was examined in 24 men. The 12 Finnish men were aged 21–40 years (30 ± 2.0). Their mean body weight was 73 ± 1.6 kg (range 63–83), and body mass index 22.3 ± 0.5

(range 19.2–24.5). The 12 Chilean subjects were aged 20–29 years (24 ± 0.9). Their mean body weight was 71 ± 3.1 kg (range 57–96 kg), and body mass index 24.0 ± 1.0 (range 19.9–31.3). None of the Finnish subjects was overweight. Three Chilean subjects were slightly overweight (body mass index 25–30), and one was obese (body mass index 31.3, height 175 cm, weight 96 kg).

All subjects were judged to be normal as assessed through medical history, general physical examination and clinical chemistry measures including blood count, liver and kidney function tests, lipid profile and assay of circulating concentrations of prostate-specific antigen. None of the subjects studied had any personal or family history of prostate cancer nor any other contraindications for androgen administration. None had been using any regular medication and none obtained any medication during the study. The pre-admission serum testosterone, LH and FSH concentrations of all subjects were within normal limits except for two subjects in study 2 who had pre-admission FSH concentrations slightly under the normal limit of 1.0 U/l (a Finnish subject in the 4 mg group had a baseline FSH concentration of 0.8 U/l, and a Chilean subject in the 2 mg group had a baseline FSH concentration of 0.6 U/l).

The studies were conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki. The ethics committees of both clinics and the institutional review board of the Population Council approved the studies. All subjects gave written informed consent before enrolment. All subjects completed the trials.

Design of the study

MENT acetate was supplied by SRI International, Menlo Park, CA, USA, and hydrolysed to MENT at the Population Council's Laboratory in New York, USA. An aqueous suspension of micronized MENT was used for the i.m. injections. All doses were given in an injection volume of 0.5 ml. In Finland the injections were given deep in the deltoid muscle and in Chile in the gluteus muscle. Blood samples were collected from an i.v. catheter fitted into a superficial vein on the back of the hand. The blood samples were allowed to clot and the sera were separated by centrifugation and stored at –20°C in plastic test tubes until assayed.

For study 1, the subjects in each clinic were divided into three groups of three. Each subject in the first group was given 2 mg of MENT. Subjects in the second and third groups received 4 and 8 mg of MENT respectively. The first blood sample was collected just before injection, the following 10 samples exactly 15, 30, 60, 90, 120, 180, 240, 300, 360 and 480 min after injection and the next samples 1, 2, 3, 4 and 9 days after injection. Blood samples for clinical chemistry measures including blood count, liver and kidney function tests, lipid profile [total cholesterol, high density lipoprotein (HDL)-cholesterol, low density (LDL)-cholesterol and triglycerides], and assay of prostate-specific antigen were obtained before treatment and 4 and 9 days after injection.

For study 2, the subjects in each clinic were divided into three groups of four. Each subject in group 1 was given 1 mg of MENT each morning for 6 consecutive days. Subjects in groups 2 and 3 received injections of 2 mg and 4 mg according to the same schedule. Blood samples were drawn before treatment and each day just before the MENT injection and thereafter at the same time in the morning 2, 3, 5, 9, 13 and 24 days after the last injection. Blood samples for SHBG assays and clinical chemistry measures including blood count, liver and kidney function tests, lipid profile (total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides), and assay of prostate-

specific antigen were obtained pre-admission, and 2 and 24 days after the last injection.

Assays

Concentrations of MENT were measured at The Population Council by radioimmunoassay as described previously (Kumar *et al.*, 1990). The detection limit of the assay was 28 pg/ml, its intra-assay coefficient of variation (CV) 7.0% and its interassay CV 13.8%. The cross-reactivity of testosterone in the MENT radioimmunoassay was 1.1%. The concentrations of testosterone, FSH, LH and SHBG were measured in the Steroid Research Laboratory, Institute of Biomedicine, University of Helsinki, Finland. Serum testosterone concentrations were determined by conventional RIA according to a standard operating procedure from the World Health Organization (WHO; Sufi *et al.*, 1990). The limit of detection was 0.5 nmol/l, the intra-assay CV 6.8% and the interassay CV 13.3%. The cross-reactivity of MENT in the testosterone radioimmunoassay was 1.2%. The concentrations of FSH, LH and SHBG were measured by time-resolved fluoroimmunoassays using commercially available kits (DELFIA®, Wallac OY, Turku, Finland). The limit of detection for LH was 0.05 IU/l, the intra-assay CV 5.0% and the interassay CV 11.7%. The limit of detection for FSH was 0.05 IU/l, the intra-assay CV 3.4% and the interassay CV 4.9%. For SHBG, the limit of detection was 0.8 nmol/l, the intra-assay CV 8.9% and the interassay CV 7.0%. All hormone and SHBG measurements were made in duplicate, and duplicates with a CV >15% were re-assayed. The other clinical chemistry assays were carried out in local laboratories.

Pharmacokinetic calculations and statistical analysis

We used the pharmacokinetic calculation methods described by Rowland and Tozer (1989), and Greenblatt and Koch-Weser (1975). To estimate the half-life of MENT after i.m. administration an exponential equation in the form $c = c_0 \times e^{-kt}$ was fitted to the combined concentration data from all subjects using a least squares fit (c is the concentration at time t, c_0 is the concentration extrapolated to zero time, e is the base of natural logarithms, k is the elimination rate constant, and t is time.) The elimination half-life was calculated by dividing the natural logarithm of 2 by the elimination rate constant. The areas under the concentration–time curves (AUC) were computed by the linear trapezoidal rule from 0 to 24 h. The plasma clearance values were calculated by dividing the doses by the AUC values.

Analysis of variance was used to test whether there were significant differences between clinics and between dose groups in pharmacokinetic parameters and basal hormone concentrations. Significant variations over time in hormone concentrations were evaluated by analysis of variance for repeated measures. For the comparison of the maximal effect of MENT on each hormone, the relative change was calculated by subtracting from each hormone concentration the baseline value of the same subject and dividing the difference by the baseline value. For each subject, the greatest relative change was identified for each hormone. The purpose of this transformation was to remove the effect of between-subject variations in baseline hormone concentrations. The maximal relative changes were compared using analysis of variance. In all statistical tests, $P < 0.05$ was considered statistically significant. All results are given as the mean ± SE.

Results

Study 1: single intramuscular injection of MENT

Serum MENT concentrations following single i.m. injections of MENT are shown in Table I. MENT concentrations in

Table I. Serum 7α-methyl-19-nortestosterone (MENT) concentrations (ng/ml) after a single i.m. injection by clinic and by dose group

Time after injection	Finnish subjects			Chilean subjects		
	2 mg	4 mg	8 mg	2 mg	4 mg	8 mg
15 min	4.6 ± 1.4	8.6 ± 3.5	14.2 ± 3.8	6.7 ± 2.4	10.7 ± 5.0	12.1 ± 4.2
30 min	5.7 ± 1.6	9.3 ± 3.1	15.5 ± 2.6	8.4 ± 2.1	11.0 ± 4.5	17.9 ± 8.4
60 min	4.9 ± 1.1	9.2 ± 4.3	14.3 ± 3.1	7.6 ± 1.0	10.4 ± 3.2	15.5 ± 6.3
90 min	4.2 ± 0.8	7.5 ± 2.5	12.8 ± 3.1	7.2 ± 0.8	8.8 ± 2.1	15.5 ± 6.6
2 h	3.9 ± 0.8	7.6 ± 1.3	11.2 ± 3.1	5.6 ± 0.5	8.3 ± 1.6	18.3 ± 8.6
3 h	2.9 ± 0.5	7.9 ± 1.4	9.0 ± 2.0	4.8 ± 0.7	8.6 ± 1.5	18.9 ± 6.7
4 h	1.6 ± 0.1	5.2 ± 0.4	7.0 ± 2.0	2.5 ± 0.6	5.7 ± 1.1	17.3 ± 6.9
5 h	1.2 ± 0.1	3.2 ± 0.5	6.4 ± 1.7	1.1 ± 0.3	3.1 ± 0.7	13.0 ± 4.0
6 h	1.1 ± 0.2	3.2 ± 0.7	6.0 ± 1.6	0.7 ± 0.1	3.2 ± 0.9	9.6 ± 3.6
8 h	0.8 ± 0.2	2.6 ± 0.9	4.2 ± 0.9	0.1 ± 0.1	1.3 ± 0.4	6.7 ± 1.5
24 h	0.0 ± 0.0	0.4 ± 0.2	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2

Values are mean ± SE.

Table II. Pharmacokinetic parameters of 7α-methyl-19-nortestosterone (MENT) after a single i.m. injection by clinic and by dose group

Subjects by clinic	Dose group	c_{max} ng/ml	t_{max} min	AUC (ng·min/ml)	(l/day)
Finnish subjects	2 mg	5.8 ± 1.5	50 ± 20	1 540 ± 170	1 910 ± 190
	4 mg	11.5 ± 3.3	90 ± 46	4 030 ± 640	1 500 ± 220
	8 mg	17.0 ± 2.6	35 ± 13	6 140 ± 1 300	2 120 ± 570
Chilean subjects	2 mg	8.9 ± 1.7	50 ± 20	1 610 ± 300	1 930 ± 380
	4 mg	12.5 ± 3.8	75 ± 53	3 360 ± 660	1 870 ± 420
	8 mg	20.0 ± 7.8	160 ± 20	9 880 ± 3 070	1 390 ± 360
Average of both clinics	2 mg	7.3 ± 1.2	50 ± 13	1 580 ± 160	1 920 ± 190
	4 mg	12.0 ± 2.3	83 ± 31	3 700 ± 440	1 690 ± 230
	8 mg	18.5 ± 3.7	98 ± 30	8 010 ± 1 710	1 750 ± 340

Values are mean ± SE. c_{max} = peak serum concentration, t_{max} = time to peak serum concentration, AUC = area under the concentration–time curve. The mean clearance for all 18 subjects was 1790 ± 140 l/day.

all samples taken after the 24 h samples were below the detection limit of the assay. The pharmacokinetic parameters are shown in Table II. There were no statistically significant differences in the pharmacokinetic parameters between the two clinics, and hence we combined the data of each dose group from both clinics. The peak MENT concentrations (c_{max}) were dose-dependent and the differences between the three dose groups were significant ($P = 0.028$). Over the 4-fold dose range tested, each doubling of the dose of MENT resulted in an increase of ~60% in c_{max}. The time to reach peak values (t_{max}) appeared to be longer at the higher doses, but the differences between dose groups were not significant. The values for AUC were dose-dependent, and the differences between dose groups were significant ($P = 0.002$). The differences in clearance rates between dose groups were small and not significant, and therefore a mean clearance rate was calculated for all subjects. The mean clearance rate was 1790 ± 140 l/day. To estimate the elimination half-life of MENT after a single i.m. injection, we combined the concentration versus time data of all subjects. The data fitted well with the exponential equation $c = c_0 \times e^{-kt}$, and the estimated elimination half-life was 224 min.

The concentrations of testosterone, LH and FSH after the injection of single doses of MENT followed the circadian patterns of these hormones (data not shown).

Study 2: six daily intramuscular injections of MENT

The concentrations of MENT in the serum samples taken during this part of the study were all below the detection limit of the assay. This is consistent with the observation in study 1 that serum concentrations of MENT were undetectable or almost undetectable 24 h after injection.

Serum testosterone concentrations in each dose group from each clinic are shown in Figure 1. The baseline testosterone concentrations in the Finnish subjects were significantly lower than those in the Chilean subjects (15.5 ± 1.2 versus 20.6 ± 1.7 nmol/l, $P = 0.029$). The decrease in testosterone concentrations after the i.m. injections was significant ($P < 0.001$). The mean of the maximal relative decrease in testosterone concentrations was dose-dependent: 41 ± 4, 59 ± 3 and 74 ± 5% in the groups who received the 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant ($P < 0.001$).

Serum LH concentrations by dose group and clinic are presented in Figure 2. There were no significant differences in baseline LH concentrations between clinics. The decrease in LH concentrations after the i.m. injections was significant
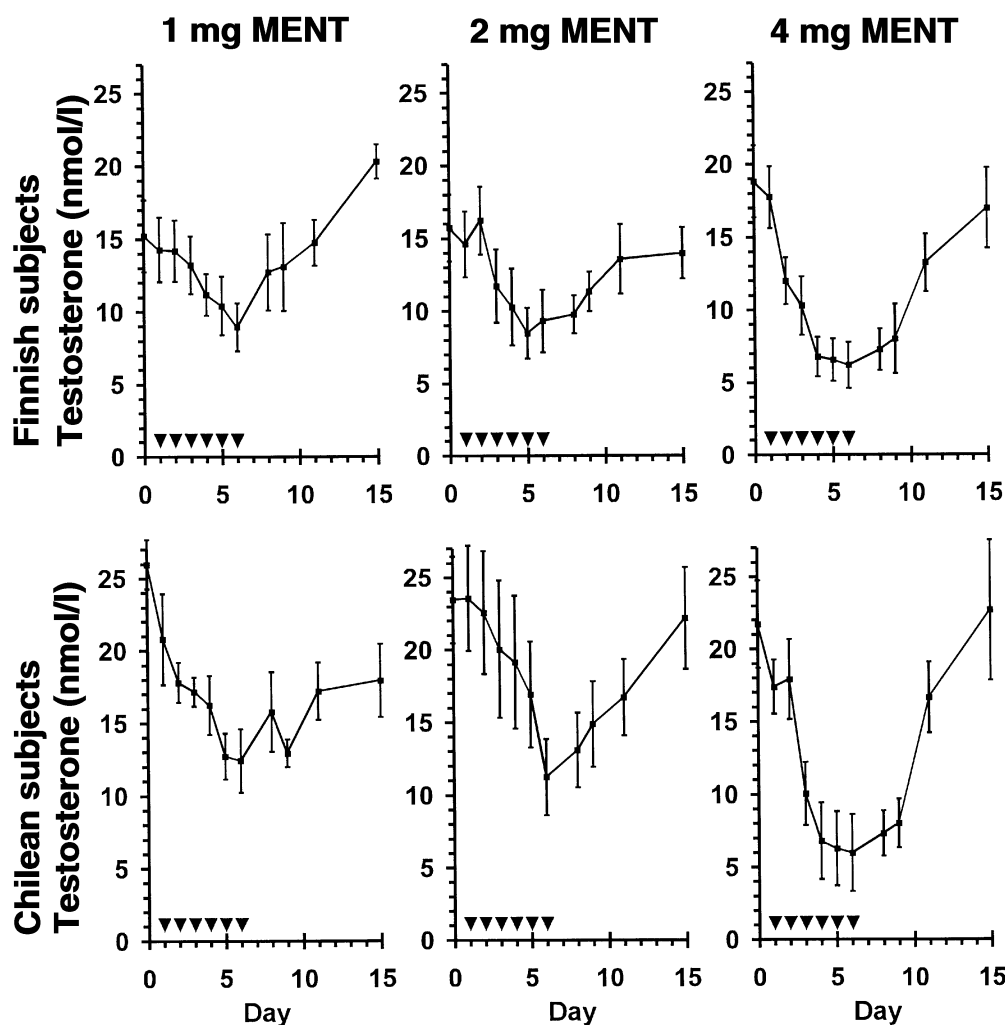
Figure 1. Mean (± SE) serum testosterone concentrations in the subjects of study 2 before, during, and after the series of six consecutive daily i.m. 7α-methyl-19-nortestosterone (MENT) injections by dose group and by clinic (*n* = 4 per group). The black triangles indicate the six injection days (days 1–6). Day 0 samples were taken before enrolment, and day 1 samples were taken just before the first injection.

(*P* < 0.001). The mean of the maximal relative decrease in LH concentrations was dose-dependent: 52 ± 3, 62 ± 6 and 70 ± 5% in the groups who received 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant (*P* = 0.048).

Serum FSH concentrations in each dose group of each clinic are shown in Figure 3. The baseline FSH concentrations in the Finnish subjects were significantly higher than those of the Chilean subjects (3.9 ± 0.4 versus 2.4 ± 0.2 U/l, *P* = 0.009). The decrease in FSH concentrations after the i.m. injections was significant (*P* < 0.001). The mean of the maximal relative decrease in FSH concentrations was dose-dependent: 25 ± 5, 41 ± 5 and 57 ± 4% in the groups who received 1, 2 or 4 mg doses, respectively. The differences between dose groups were statistically significant (*P* = 0.001).

By day 15, nine days after the last injections, all hormone concentrations had returned to near their baseline values and the concentrations in the last two samples were similar; hence the last two samples are not shown in the Figures.

In addition to the decreases in hormone concentrations, clear decreases in SHBG concentrations were also observed. The mean serum SHBG concentration of all 24 volunteers decreased

from 31 ± 2.2 (baseline) to 26 ± 2.1 nmol/l (two days after the last injection). This decrease in SHBG concentrations after the i.m. injections was significant (*P* < 0.001). There were no significant differences either in baseline concentrations or in the mean decreases between clinics or between dose groups.

Clinical chemistry and safety

The subjects did not report any adverse effects. The total neutrophil count decreased to 1.7×10^9/l in two Finnish subjects in study 2. Total serum cholesterol in one Finnish subject in study 1 increased from 4.7 to 6.3 mmol/l, and his LDL-cholesterol concentration increased from 2.6 to 4.5 mmol/l. In addition, three other Finnish subjects in study 1 and three Finnish subjects in study 2 had increases in total serum cholesterol from desirable values to borderline high values. In study 1, two Finnish subjects and one Chilean had clear increases in their triglyceride concentrations. The greatest increase was from 0.9 to 3.3 mmol/l. In study 1, one Finnish subject had a small increase in aspartate aminotransferase (AST) concentrations, while another had a small increase in alanine aminotransferase (ALT) concentrations. Lactate dehydrogenase (LD) increased slightly in three Chilean
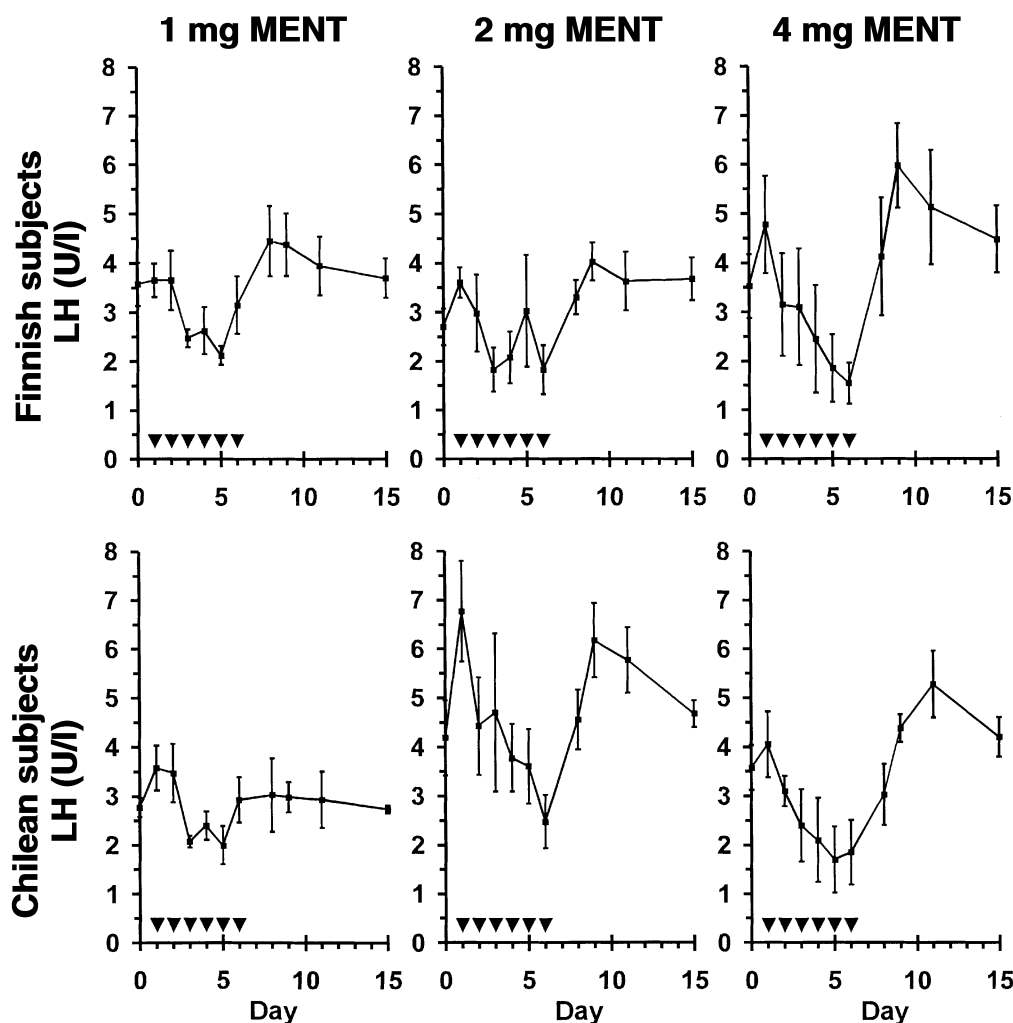
Figure 2. Mean ($\pm$ SE) serum luteinizing hormone (LH) concentrations in the subjects of study 2 before, during, and after the series of six consecutive daily i.m. 7α-methyl-19-nortestosterone (MENT) injections by dose group and by clinic (see legend to Figure 1 for details).

subjects. In study 2, one Finnish subject had an increase in AST from 27 to 72 U/l and an increase in LD from 351 to 530 U/L (upper limit, 450 U/l). In addition, another Finnish subject and three Chilean subjects also had increases in LD. The highest increase in LD among the Chileans was from 175 to 383 U/l, (upper limit, 225 U/l). The same subject also had an increase in AST from 28 to 123 U/l (upper limit, 37 U/l) (note the different upper limits of several liver function test results in Finland and Chile. This is due to differences in laboratory methods). In both clinics and both studies, the serum concentrations of prostate-specific antigen remained well below the upper limit of the reference range (4 μg/l).

Discussion

We here report the first study on the pharmacokinetics of MENT given i.m. to healthy men. The pharmacokinetics of MENT given i.v. have been studied previously (unpublished results). The mean clearance rate after i.m. administration (1790 $\pm$ 140 l/day) was not very different from the clearance rate determined following i.v. administration, 2100 $\pm$ 160 l/day. In contrast, the half-life estimated from the data of the present study (224 min) was more than five times longer

than the 40 min elimination half-life determined using a single i.v. dose of 0.5 mg MENT. This difference is almost certainly explained by the slow absorption of MENT from the i.m. injection site (Rowland and Tozer, 1989). If absorption is slow enough, it is rate-limiting in the decay phase of MENT concentrations, and the estimated half-life corresponds to the absorption half-life. Since MENT is poorly soluble in water, its absorption from muscle could be relatively slow (Greenblatt and Koch-Weser, 1975). The striking difference in the t_{max} values between the 8 mg groups in Finland and in Chile is explained by the fact that one subject in Finland had the peak concentration of MENT in the first sample collected (15 min after the injection). The cause is unclear, but the drug may have been injected in the vicinity of a vessel, leading to rapid absorption.

The changes in the serum concentrations of testosterone, LH and FSH after the single i.m. MENT injections were not reported, since in the absence of a placebo group, the possible effect of MENT on these concentrations cannot be distinguished from natural circadian variation (Wisser and Breuer, 1981; Bridges *et al.*, 1993). On the other hand, it is important to note that circadian variation could not influence the results of the study of six daily MENT injections, since in
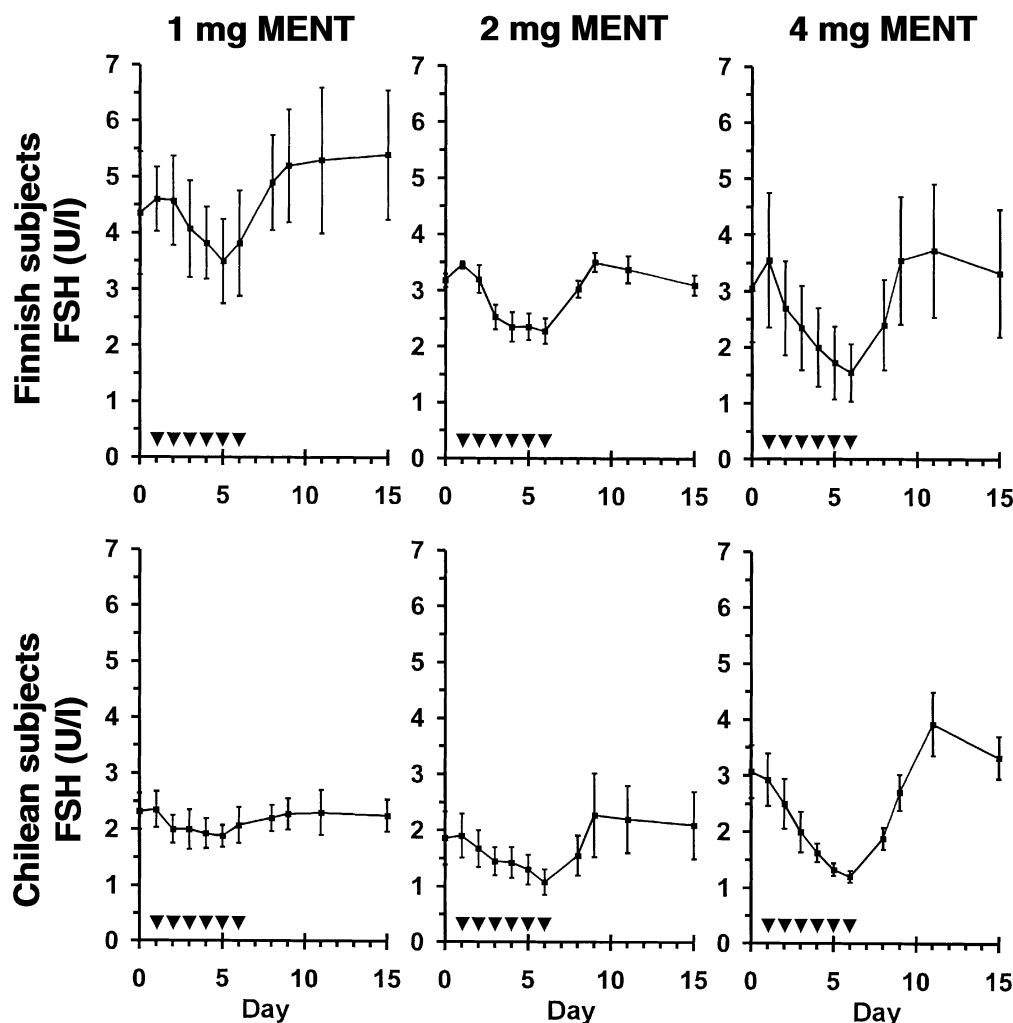
Figure 3. Mean ($\pm$ SE) serum follicle stimulating hormone (FSH) concentrations in the subjects of study 2 before, during, and after the series of six consecutive daily i.m. 7α-methyl-19-nortestosterone (MENT) injections by dose group and by clinic (see legend to Figure 1 for details).

this study, all blood samples for hormone assays were taken at the same time in the morning.

The deviations from reference ranges observed in some clinical chemistry parameters were small and did not follow a clear trend or pattern suggestive of a serious adverse effect. However, the changes in the lipid profile and liver function test results should be taken as a signal to pay careful attention to the monitoring of these parameters in future studies since similar changes have been associated with androgen administration in earlier studies. No reliable conclusions on the safety of MENT can be drawn from these small phase I clinical studies.

The Finnish volunteers who participated in the study of six MENT injections had significantly lower baseline testosterone concentrations and significantly higher baseline FSH concentrations than the Chilean volunteers. It is likely that this is a true difference, since all hormone concentrations were measured in the same laboratory, and the control samples did not reveal any significant between-assay variation in testosterone or FSH measurements. A possible cause for this difference is the fact that the Finnish subjects were significantly older than the Chileans (30 $\pm$ 2.0 versus 24 $\pm$ 0.9 years, $P = 0.009$).

A significant decline with age in circulating testosterone concentrations has been described (Dabbs, 1990). There may also be true differences between these two ethnic groups in hormone concentrations. Still another possible explanation is that since Finland is situated in the northern hemisphere and Chile in the southern, seasons are opposite, and seasonal variation in the concentrations of testosterone and FSH could influence the results.

MENT has previously been shown to be a potent androgen in animals and to have antigonadotrophic effects (Kumar *et al.*, 1992). In humans, the androgenicity of MENT has been demonstrated by its masculinizing effects in female breast cancer patients (Segaloff *et al.*, 1964; O'Brian 1966). In these early studies, a pituitary gonadal response to MENT could not be shown directly, since methods for the assay of serum gonadotrophin concentrations were unsatisfactory at that time. Urinary excretion of gonadotrophins was measured and although a tendency toward a decrease in gonadotrophin excretion was observed, these changes were not consistent.

Our study is the first to demonstrate that MENT is a potent antigonadotrophic agent in men. In the study of six consecutive i.m. injections a biological response typical of exogenous

androgen administration was observed: serum testosterone, LH, FSH and SHBG concentrations decreased significantly during the injection series. Over the dose range studied, there was also a clear dose–response relationship, with the exception of SHBG concentrations. This study supports the idea that small daily doses of MENT could be sufficient for androgen replacement. The suggested route of MENT administration is via subdermal implants (Sundaram *et al.*, 1993). Future studies with implants will show whether this approach can provide sufficient androgen replacement for a reasonable time.

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