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SHORT COMMUNICATION

Testosterone disposition after intramuscular injection in castrated thoroughbred race horses

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Castration is a frequently performed surgery in thoroughbred race horses. Hormonal restitution therapies have been proposed and tested in castrated males in different animal species, including bulls (Haynes *et al.*, 1976) and humans (Snyder & Lawrence, 1980), but no information on horses is available. Testicular endocrine function has been shown to be important in the regulation of erythropoiesis and haemoglobin synthesis (Naets & Wittek, 1966; Minguell & Sierraila, 1975; Perretta *et al.*, 1982), in muscular tissue synthesis (Powers & Fiorini, 1975) and skeletal development, in improving aggressiveness, strength and stamina (Dawson & Gersten, 1978; Beroza, 1981), as well as being involved in muscle glycogen supercompensation (Gillespsie & Edgerton, 1970). Plasma testosterone concentration in a normal stallion varies between 0.22 and 5.54 nmol/l (Cox *et al.*, 1973). Castration leads to a sharp decrease in these concentrations, being reported to be less than 1.04 nmol/l 12 h after surgery (Gamjam & Kenney, 1975). Similar observations have been reported in dogs, where plasma testosterone concentrations are undetectable 48 h after castration (Taha *et al.*, 1981).

Testosterone may play an important role in the locomotive performance in thoroughbred race horses, but negative metabolic effects have been reported in over-dosage conditions (Dawson & Gersten, 1978; Beroza, 1981). In order to objectively define doses and administration intervals for testosterone restitution therapy, it is important to define the castration effect on endogenous plasma testosterone concentration and to determine the pharmacokinetic parameters in a restitution therapy using the most common doses applied at random in equine race practice.

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Eight thoroughbred race horses, between 2 and 8 years of age, 430–500 kg body weight, were used. Animals were in full training practice, under usual management and feeding schemes. No other medication besides testosterone enanthate was given during the study period. One week after castration, testosterone enanthate (kindly donated by Laboratorio Chile S.A.) was injected i.m. in single doses of 0.50, 1.00, 1.25 and 1.50 g, leaving an approximately one-month interval between each dose (28–32 days). Blood samples were obtained at 8 a.m., by jugular venipuncture using evacuated blood collection tubes with ethylendiaminetetraacetic acid (EDTA) as an anticoagulant. An initial sample was obtained 15 min before castration to evaluate normal testosterone concentration. Basal residual testosterone concentrations were estimated one week after castration. Post-treatment sampling was as frequent as possible, considering horse racing regulations, being no less than one day and no more than six days between samples. Plasma was obtained within two hours after blood collection and kept frozen until testosterone analysis was performed.

Plasma testosterone concentration was assayed by radioimmunoassay (RIA) using reagents and procedures provided by the World Health Organization (WHO, 1983). The assay was a conventional liquid phase RIA for total plasma/serum testosterone, including ether extraction, tritiated testosterone as tracer and dextran-charcoal for separation of free from antibody-bound hormone fraction. As the technique was developed to

estimate total testosterone in human plasma or serum, it was validated for equine plasma prior to its use. The intra-assay and inter-assay coefficient of variation percentages (CV%) were 4.0% and 15.3% in low level quality control (QC) samples (1.5 nmol/l), and 7.5% and 8.9% in high level QC samples (19.7 nmol/l), respectively. The assay sensitivity was 0.1 pmol/tube (at CV% = 10).

The elimination rate (K_e) calculated from the slope of the terminal portion of log plasma testosterone concentration *vs.* time plot was determined through linear regression analysis. The area under the plasma testosterone concentration *vs.* time plot from time zero to t (AUC_{0-t}) was calculated using the linear trapezoidal method. The area from time t to infinity ($AUC_{t-\infty}$) was estimated by C_n/K_e, where C_n is the concentration at the last measured time point. Mean residence time after intramuscular doses of testosterone enanthate ($MRT_{i.m.}$) was calculated by $MRT_{i.m.} = AUMC_{0-\infty}/AUC_{0-\infty}$, where $AUMC_{0-\infty}$ is area under the first moment of plasma testosterone concentration *vs.* time curve (Yamaoka *et al.*, 1978).

Since absorption rate was not readily obtained due to the presence of multiple absorption maxima (especially in the case of the 0.50-g dose) and insufficient plasma testosterone concentration data in the initial time points, an uncorrected mean absorption time was calculated as $MAT_{uncorr.} = MRT_{i.m.} - 1/\lambda_n$ where λ_n is the respective slope of the terminal log-linear phase (Riegelman & Collier, 1980; Jackson & Chen, 1987).

Differences between *AUC*s, K_e and *MAT* were assessed by ANOVA as indicated by Wagner (1975) and Westlake (1973, 1979) with statistical significance taken as $P < 0.05$. When significant differences were found, Dunnett's multiple range test was applied to identify where those differences occurred (Dunnett, 1964; Wagner, 1975).

Basal plasma testosterone concentration 15 min before castration was 2.04 ± 1.55 nmol/l (CV = 75.9%). This high degree of variation was similar to that reported by others (Cox *et*

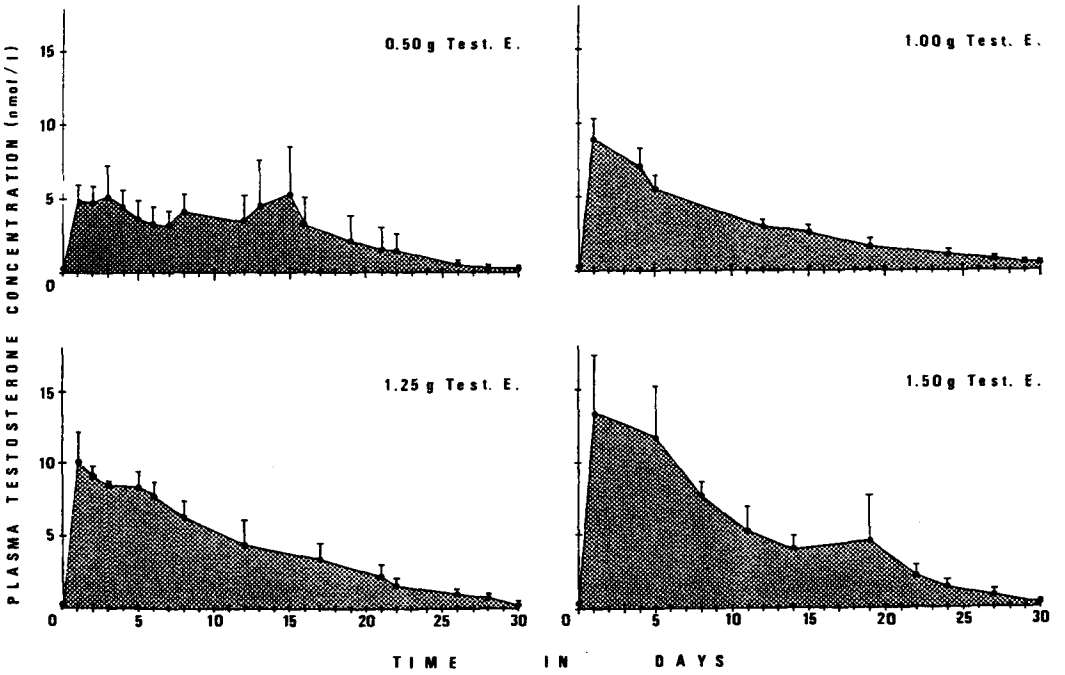

FIG. 1. Plasma concentration profiles of testosterone *vs.* time in eight castrated horses treated with testosterone enanthate at different single doses (i.m.).

TABLE I. Pharmacokinetic parameters of testosterone in horses

Dose (g)	K_e (day^{-1})	$t_{1/2}$ (day)	*AUC* (nmol × day/l)	*AUC*/dose (nmol × day/l × g)	*MRT* (day)	*MAT* (day)
0.50	0.132 ± 0.072 (54.545)*	6.120 ± 2.082 (34.014)	73.183 ± 19.911 (27.207)	146.272 ± 25.34 (17.328)	10.359 ± 1.798 (17.354)	1.362 ± 1.243 (91.262)
1.00	0.095 ± 0.008 (8.771)	7.324 ± 0.712 (9.722)	100.097 ± 8.623 (8.615)	100.097 ± 8.623 (8.615)	9.139 ± 0.587 (6.422)	1.316 ± 0.439 (33.358)
1.25	0.109 ± 0.019 (17.099)	6.528 ± 1.065 (16.322)	129.966 ± 13.566 (10.438)	103.972 ± 10.853 (10.439)	9.644 ± 1.000 (10.377)	0.593 ± 0.415 (69.983)
1.50	0.128 ± 0.010 (7.995)	5.444 ± 0.437 (8.022)	162.752 ± 34.241 (21.039)	108.497 ± 22.831 (21.043)	8.740 ± 0.827 (9.468)	1.171 ± 0.752 (64.219)

K_e = elimination rate constant; $t_{1/2}$ = elimination half time; *AUC* = area under the curve of plasma concentration *vs.* time (area from time zero to ∞); *MRT* = mean residence time; *MAT* = mean absorption time; * = coefficient of variation (%).

al., 1973; Cox & Jawad, 1979). Values after castration were significantly different from basal levels in all cases studied, decreasing to 0.31 ± 0.17 nmol/l (CV = 54.8%) ($P < 0.01$; *t*-test). Fig. 1 shows the mean plasma testosterone concentration *vs.* time in eight horses. It can be observed that absorption from the intramuscular depot of testosterone enanthate is high and effective levels of the hormone are detected until approximately 4 weeks, particularly with the 1.0-g dose of hormone. Table I shows the results of the mean pharmacokinetic parameters of testosterone in horses. The elimination rate, as estimated by K_e, was not significantly different between doses using ANOVA ($P > 0.05$), indicating that elimination rate is independent of dose over the ranges employed in this study.

The *MAT* values obtained indicate a rapid absorption of the drug. This absorption shows a great variation among animals, revealed by the high coefficient of variation. Absorption rates were independent of dose by ANOVA ($P > 0.05$). ANOVA results for *AUC*s indicate no statistical differences exist among animals or periods of administration ($P > 0.05$), although significant differences were found between treatments (doses). Dunnett's multiple range test revealed that statistical differences exist between all doses, excepted the lowest (0.5 g). This is also supported by the *AUC* proportionality and normalization data (Table I). Only the ratio of *AUC*/dose for the 0.50-g dose is higher than the others. This is probably due to analytical errors in testosterone determination due to the low plasma concentration. These results indicate that the extent of the absorption from the intramuscular depot of testosterone enanthate appears to be linear to the dose injected, as is shown in Fig. 2.

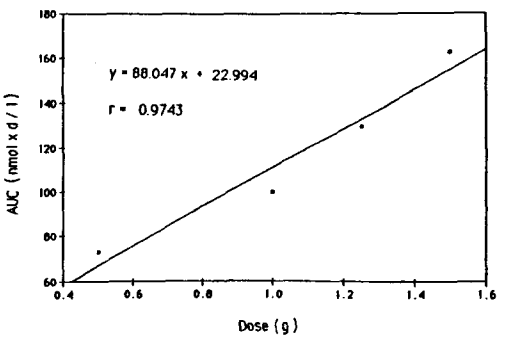

FIG. 2. Linearity between *AUC* and dose of testosterone enanthate in horses.

The 0.50-g dose of testosterone enanthate could be objectively recommended for restitution therapy in order to obtain hormonal plasma concentrations close to normal for about 19 days. Higher doses of the drug would not significantly increase the covering period and could induce hepatic metabolic disorders.

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