Hypocholesterolemic activity of 17α-methyl-5α-androstane-3β,17β-diol, a metabolite of 17α-methyltestosterone

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ABSTRACT The hypocholesterolemic activities of 17α-methyltestosterone and its major identified fecal metabolite, 17α-methyl-5α-androstane-3β,17β-diol, were compared in dogs. The dose–response curves indicated that the two compounds had similar effects, although at doses below 1 mg/kg per day the diol appeared to be more active than the parent compound.

KEY WORDS 17α-methyltestosterone · 17α-methyl-5α-androstane-3β,17β-diol · hypocholesterolemic agent · dog · serum cholesterol · serum lipoprotein

In the first paper of this series (1) we reported studies on the metabolic disposition of 17α-methyltestosterone in dogs. Four isomeric methylandrostanediols were isolated from the feces and identified. The present study describes the hypocholesterolemic activity in dogs of the predominating fecal diol, 17α-methyl-5α-androstane-3β,17β-diol.

MATERIALS AND METHODS

The 13 adult mongrel dogs (7 males and 6 females) used throughout the experiments were housed in individual cages and maintained on a diet of commercial dog biscuits (Big Red Kibbled Dog Cakes, GFL Marketing Co., Canandaigua, N.Y.). The dogs ranged in weight from 2.7 to 14.1 kg; each dog was weighed weekly and showed no significant weight changes during the experimental period. After a 2–3 wk control period appropriate quantities of methyltestosterone or of the metabolite were administered five times per week, usually for 4 wk, either by mouth in gelatin capsules or intramuscularly. When less than 20 mg/day was fed, the drug was triturated with finely ground dog biscuits and the appropriate amount incorporated into the capsules. The vehicle for the intramuscularly administered drugs was a mixture of sesame oil, benzyl benzoate, and benzyl alcohol, 34:15:1 by volume.

All dogs were bled weekly during the control and experimental periods as well as during a 4 wk "post-treatment control" period. Sera were analyzed for total cholesterol either by the method of Abell, Levy, Brodie, and Kendall (2) or by the AutoAnalyzer technique, method N 24a (3). These methods gave identical results within the precision of measurement. At 2-wk intervals the sera were fractionated in the preparative ultracentrifuge (spinco Model L) at a specific gravity of 1.063. The low density (LDL) and high density (HDL) lipoprotein fractions were isolated according to the method of Havel, Eder, and Bragdon (4) and analyzed for total cholesterol as previously described (5).

RESULTS

Table 1 lists the serum cholesterol concentrations of the dogs that received methyltestosterone orally in doses

This is paper 2 of a series on the metabolism of 17α-methyltestosterone in the dog. Paper 1 is the preceding paper (ref. 1).

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Abbreviations: HDL, high density lipoprotein fraction; LDL, low density lipoprotein fraction.

ranging from 18 mg/kg to 0.1 mg/kg, together with the percentage decreases in cholesterol level at the end of treatment. The values given for the control period represent the average of two or three weekly determinations and the arithmetic variation. The differences in serum cholesterol levels noted during the control period are within expected biological variation. Such fluctuations are random and are ordinarily smaller than the changes produced by the drugs used in the present study. The values for total cholesterol listed next show the mean of the values obtained after 3 and 4 wk of drug treatment and the arithmetic variation. After 1 wk of treatment a 20% average decrease in serum total cholesterol levels was noted in six out of the nine dogs studied at this time. At the end of 3 wk the maximal hypocholesterolemic effect with the dosage used had usually been obtained. However, occasional exceptions were observed, especially in the low dosage range. When treatment was discontinued the cholesterol levels slowly returned toward control values during a 4 wk observation period. The last value obtained during this post-treatment control period is listed in the table. The percentage decreases effected by the drug and shown in the last column were not apparently related to the starting cholesterol levels of the animals.

Fig. 1 depicts the percentage distribution of total serum cholesterol between the HDL and LDL fractions of the sera of representative dogs listed in Table 1. Only the dosage range between 10 mg/kg and 0.1 mg/kg is covered here. Within the precision of measurement there was no change in the distribution of cholesterol between these two fractions as a result of methyltestosterone treatment.

Table 2 indicates the serum total cholesterol concen-

TABLE 1 HYPOCHOLESTEROLEMIC ACTIVITY OF ORALLY ADMINISTERED 17α-METHYLTESTOSTERONE IN DOGS

Dog	Weight	Dose	Serum Cholesterol			
			Control Period*	Experimental Period†	Post-Treatment Control‡	% Decrease
	kg	*mg/kg*		*mg/100 ml*		
Sc M	11.0	18	114 ± 6	56 ± 7	127	51
Pw M	7.7	10	124 ± 10	65 ± 1	132	48
Be F	8.2	10	166 ± 6	119 ± 1	166	28
Ra F	4.6	5	140 ± 2	87 ± 6	127	38
Pw M	7.7	2	139 ± 6	82 ± 7	114	41
Be F	9.1	1	154 ± 16	80 ± 15	170	48
Bo M	8.2	0.5	100 ± 9	67 ± 1	117	33
Li M	7.7	0.5	94 ± 9	93 ± 13	123	1
Pe M	11.8	0.1	128 ± 8	121 ± 10	163	5
Be F	14.0	0.1	152 ± 5	133 ± 15	158	12

* Mean of two or three determinations ± maximum variation from mean.
† Values after 3 and 4 wk on drug ± maximum variation from mean.
‡ Value 3–4 wk after cessation of medication.

TABLE 2 HYPOCHOLESTEROLEMIC ACTIVITY OF ORALLY ADMINISTERED 17α-METHYL-5α-ANDROSTANE-3β,17β-DIOL IN DOGS

Dog	Weight	Dose	Serum Cholesterol			
			Control Period*	Experimental Period†	Post-Treatment Control‡	% Decrease
	kg	*mg/kg*		*mg/100 ml*		
Bo M	8.7	13	102 ± 15	53§	97	48
Je F	5.7	10	187 ± 8	103§	177	45
Je F	5.7	5	168 ± 9	144 ± 4	178	14
Bo M	8.7	2	88 ± 8	62 ± 3	95	30
Sp F	8.9	1	130 ± 3	84 ± 9	104	35
Be F	10.9	0.5	155 ± 11	124 ± 2	196	20
Pe M	12.3	0.5	129 ± 10	98 ± 4	120	24
Bo M	8.4	0.1	113 ± 8	66 ± 7	88	42
Li M	7.4	0.1	119 ± 7	92 ± 1	99	22

* Mean of two or three determinations ± maximum variation from mean.
† Values after 3 and 4 wk of medication ± maximum variation from mean.
‡ Value 3–4 wk after cessation of medication.
§ After 2 wk on medication.

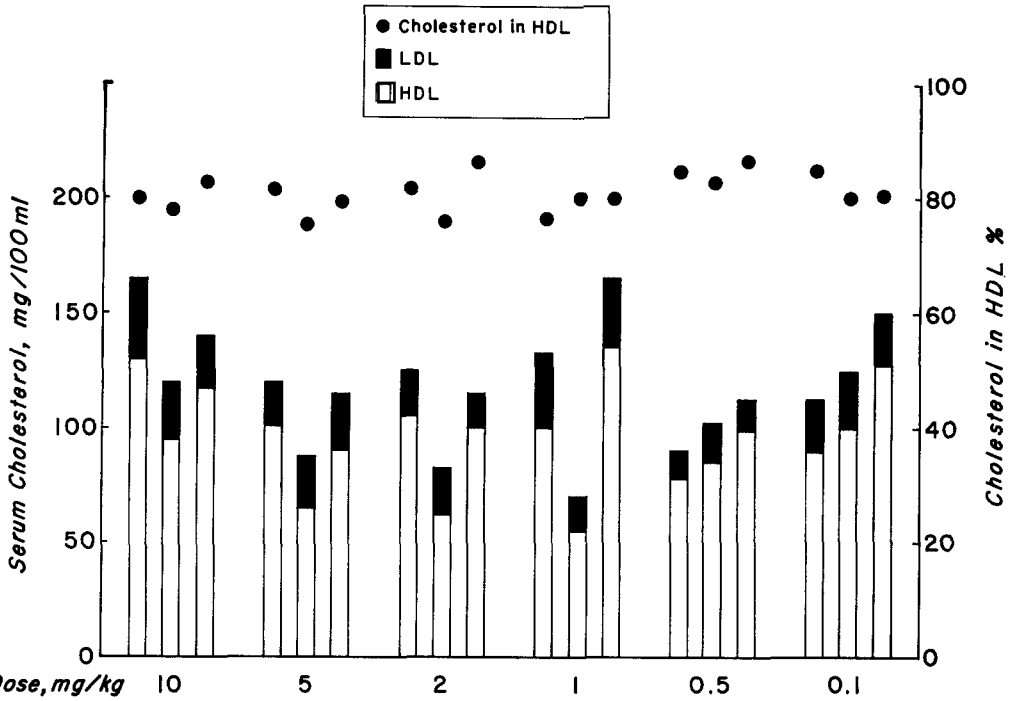

FIG. 1. Distribution of serum cholesterol between HDL and LDL fractions of dogs receiving methyltestosterone. Each group of three bars represents the serum cholesterol pattern of a single dog at the end of the control period, of the 4 wk experimental period, and of the post-treatment control period.

trations of the dogs that received 17α-methyl-5α-androstane-3β,17β-diol, the most abundant fecal metabolite of 17α-methyltestosterone, in doses ranging from 13 mg/kg to 0.1 mg/kg. The data are recorded in the same manner as those in Table 1. Upon administration of the drug there was a progressive decrease in serum cholesterol concentrations during the first 3 wk of treatment; in some instances this drop was apparent at the end of 1 wk. As in the methyltestosterone-treated dogs, the maximal hypocholesterolemic effect of the drug was noticeable after 3 wk of treatment. At the end of the 4 wk post-treatment control period the cholesterol concentration of most dogs had returned to their pretreatment levels. As in the experiment with methyltestosterone, the drop in serum cholesterol concentrations effected by the metabolite was independent of the initial levels.

Fig. 2 presents the percentage distribution of cholesterol between the HDL and LDL fractions of the sera of representative dogs listed in Table 2. There is very little change in this parameter as a result of drug administration.

Fig. 3 presents experiments in which the metabolite was injected intramuscularly into two dogs, five times per week, over a 4 wk period at a dose level of 1 mg/kg. For comparison methyltestosterone at the same dosage level was injected into two other dogs. The hypocholesterolemic activities were similar to those when the drug was given parenterally.

DISCUSSION

Plotting the fractional drop in serum cholesterol concentrations shown in Tables 1 and 2 against the logarithm of the dose and calculating the best regression line through these points by the method of least squares (6) showed that the dose response of the dogs was the same[1] for the oral administration of the two drugs. At dose levels above 10 mg/kg per day the administration of either 17α-methyltestosterone or 17α-methyl-5α-androstane-3β,17β-diol lowered serum total cholesterol levels about 50%. At doses between 10 and 1 mg/kg per day the hypocholesterolemic effects of the two drugs were similar: on the average, methyltestosterone produced a decrease of 41% and diol administration resulted in a decrease of 31%. At lower doses (0.5 and 0.1 mg/kg per day) the diol seemed to be more active than the parent compound; the average decreases obtained with methyltestosterone and diol were 13% and 27%, respectively.

Eder has reported (7) that the most consistent result of the administration of methyltestosterone to human beings was an increase of the cholesterol content of the LDL fraction. This increase was at times large enough to cause a *rise* in serum total cholesterol concentration. In patients on protein-free diets, Furman, Howard, and Norcia (8) found that administration of methyltestos-

[1] Methyltestosterone: y = 0.299 + 0.216 (log dosage). Diol: y = 0.275 + 0.173 (log dosage).

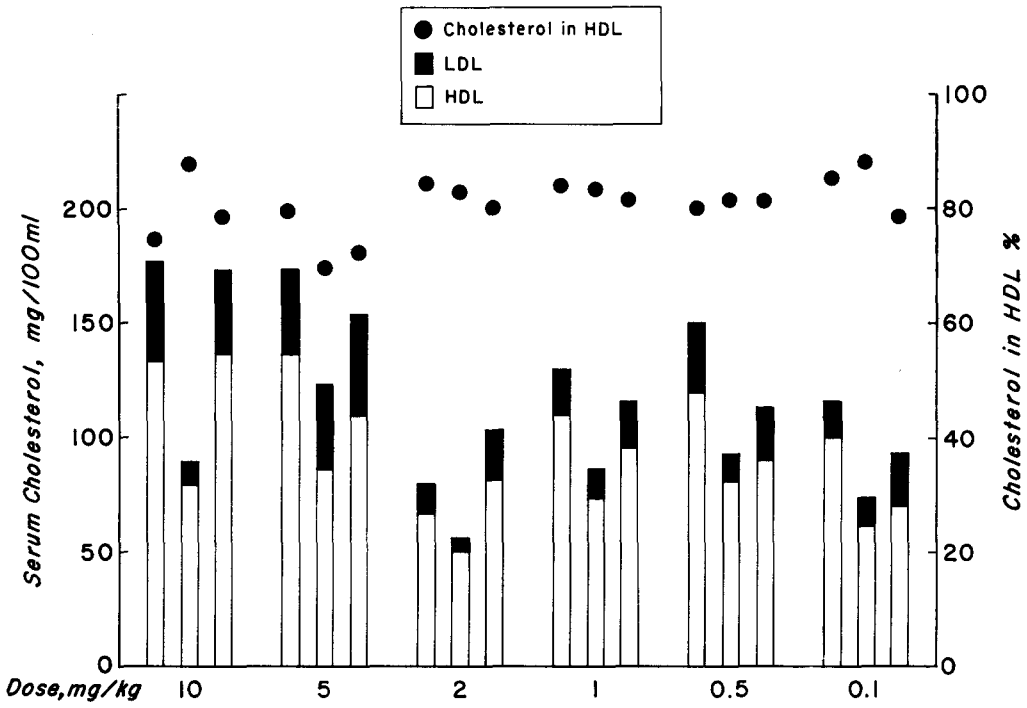

FIG. 2. Distribution of serum cholesterol between HDL and LDL fractions of dogs receiving 17α-methyl-5α-androstane-3β,17β-diol. Each group of three bars represents the serum cholesterol pattern of a single dog at the end of the control period, of the 4 wk experimental period, and of the post-treatment control period.

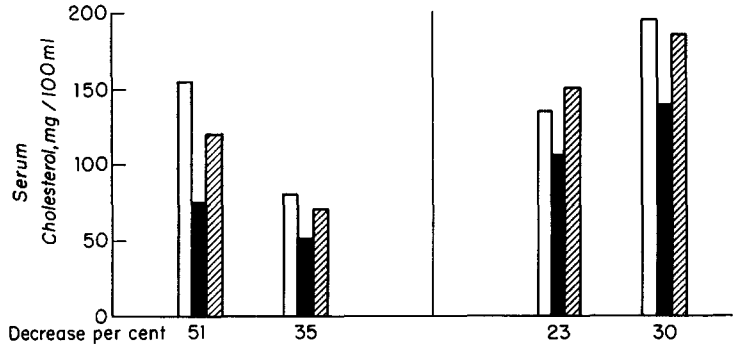

FIG. 3. Intramuscular administration (1 mg/kg) of methyltestosterone (left) and 17α-methyl-5α-androstane-3β,17β-diol (right) to dogs. The bars represent serum cholesterol concentrations. Open bars, levels at end of control period; solid bars, levels at end of treatment period; shaded bars, levels at end of post-treatment control period.

terone reduced the cholesterol content of both HDL and LDL fractions. In the present study we found that in dogs on low cholesterol diets both drugs reduced the cholesterol content of the two lipoprotein fractions to the same extent, i.e., neither methyltestosterone nor the diol produced a shift in the distribution of serum cholesterol between the LDL and HDL fractions of the sera tested. This is not surprising as it has been observed in previous studies (9) that such a shift occurs only in dogs maintained on high cholesterol diets.

Although detailed studies on the androgenic potency of 17α-methyltestosterone and its metabolites in dogs have not been published, in capons the diol studied here has less than 10% as much activity as the parent compound (10). Therefore it seems unlikely that there is a direct relationship between the androgenicity and the hypocholesterolemic activity of the steroids studied in these experiments. The high potency of the diol in reducing serum cholesterol concentrations suggests but does not prove that it may be the effective compound when methyltestosterone is fed (1). This possibility will be further investigated.

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