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TESTOSTERONE AND 4-ANDROSTENEDIONE CONCENTRATION IN PERIPHERAL AND SPERMATIC VENOUS BLOOD OF PATIENTS WITH PROSTATIC ADENOCARCINOMA

EFFECTS OF DIETHYLSTILBESTROL* AND CYPROTERONE ACETATE THERAPY†

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SUMMARY

The present investigation has been carried out in 19 patients affected by prostatic adenocarcinoma, in an effort to obtain further information on the mechanism of action of diethylstilbestrol and cyproterone acetate on the testicular androgen function.

Testosterone and 4-androstenedione concentrations were determined in peripheral and spermatic venous blood, obtained simultaneously at the time of orchiectomy.

The pre-treatment mean peripheral plasma levels of testosterone were within the normal range ($0{\cdot}446 \pm 0{\cdot}157$ μg/100 ml) while 4-androstenedione was slightly increased ($0{\cdot}169 \pm 0{\cdot}088$ μg/100 ml). Comparatively the values in spermatic venous blood were $9{\cdot}792 \pm 5{\cdot}046$ μg/100 ml for testosterone and $0{\cdot}854 \pm 0{\cdot}296$ μg/100 ml for 4-androstenedione.

After diethylstilbestrol treatment peripheral and spermatic testosterone levels decreased to $0{\cdot}128 \pm 0{\cdot}070$ μg/100 ml and to $0{\cdot}915 \pm 0{\cdot}766$ μg/100 ml respectively, whereas no significant variations were registered for 4-androstenedione.

Similar results were obtained with cyproterone acetate: reduction of testosterone concentrations ($0{\cdot}177 \pm 0{\cdot}079$ μg/100 ml in peripheral blood, $1{\cdot}800 \pm 1{\cdot}064$ μg/100 ml in spermatic vein) and no significant variations for 4-androstenedione.
atic vein) and no significant variations for 4-androstenedione.

In conclusion these data demonstrate that diethylstilbestrol and cyproterone acetate express their antiandrogenic effects on the testicular function, inhibiting mainly the secretion of testosterone, which is one of the most important hormonal factors in the growth of prostatic adenocarcinoma.

INTRODUCTION

IN PROSTATIC adenocarcinoma, a hormone dependent tumour, it is generally accepted that androgens stimulate the growth of neoplastic tissue [1, 2].

It has also been reported that large doses of estrogens may arrest the growth and even reduce the volume of the neoplasm, by inhibiting androgen production [3]. However this inactivating effect is only temporary and the prostatic carcinoma becomes an estrogen resistent tumour.

The administration of other compounds with antiandrogenic activity (e.g. progestagens and more recently cyproterone acetate*) once again inhibits the growth of the neoplastic tissue, even in cases where the estrogenic treatment was no longer effective [4–6].

However, although the usefulness of these steroids is well documented in clinical practice, little is known about their mechanism of action.

*Diethylstilbestrol diphosphate (Honran).

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In a previous paper we reported on the effects of diethylstilbestrol‡ and cyproterone acetate treatment on the testicular biosynthesis of testosterone, 4-androstenedione and dehydroisoandrosterone[7]. Evaluations were made by incubating specimens of gonadal tissue with [4-^{14}C] pregnenolone *in vitro*, and it was demonstrated that these compounds modify the biosynthetic pattern, inhibiting the transformation of the precursor into testosterone.

For a better evaluation of the testicular androgen function before and after estrogen and cyproterone acetate administration, these *in vitro* experiments need to be confirmed by *in vivo* studies.

The present investigation was undertaken to evaluate the plasma testosterone and 4-androstenedione concentration in peripheral and spermatic venous blood, obtained simultaneously at the time of orchiectomy in patients affected by prostatic adenocarcinoma.

MATERIAL AND METHODS

Solvents for plasma extraction and chromatography were spectro-grade.

Anhydrous ethyl ether RS (Carlo Erba); methylene chloride RS (Carlo Erba); benzene RS(Carlo Erba); methanol RS(Carlo Erba); toluene RP(Carlo Erba); naphthalene(Carlo Erba); sodium borohydrate(Carlo Erba); charcoal activated powder A.R.(Riedel-DeHaen AG., Seelze-Hannover); dextran (Schuchardt, München); dioxane (Merck, Darmstadt); PPO (Beckman); dimethyl POPOP (Beckman); t.l.c. aluminium sheets, aluminium oxide neutral 20 × 20; layer thickness 0·2 mm (Merck, Darmstadt); [1,2-^{3}H]testosterone (NEN), S.A. 40 Ci/mmol; [1,2-^{3}H]4-androstenedione (NEN), S.A. 40 Ci/mmol; testosterone standard (Mann Res. Laboratory, New York); 4-androstenedione standard(Mann Res. Laboratory, New York).

Plasma testosterone and 4-androstenedione levels were measured by competitive protein binding technique, based on the principles of analysis of Fritz and Knobil[8], and Kato and Horton[9], described in detail earlier by us[10]. In summary, the method is as follows:

0·1–1 ml plasma samples were extracted with ethyl ether, which was then washed with 1 N NaOH and water.

The dry residue was redissolved in a few drops of methylene chloride: methanol (9:1, v/v), chromatographed on t.l.c. and developed in the benzene: ether system (60:40, v/v). Testosterone and 4-androstenedione were eluted with methylene chloride: methanol (9:1, v/v).

The 4-androstenedione was treated for 45 min with sodium borohydride for conversion to testosterone and rechromatographed in the same t.l.c. system.

The recovery for each determination, which was calculated by adding approximately 800 cpm of [1,2-^{3}H]testosterone and 1500 cpm of [1,2-^{3}H]4-androstenedione to the plasma samples, was 75–90% for testosterone and 45–60% for 4-androstenedione.

The competitive protein binding reaction was carried out with 2% third trimester pregnancy plasma in 0·9%(w/v) saline solution, and separation of the bound testosterone from the unbound was obtained with a dextran-coated charcoal suspension.

‡In this report the following abbreviations are used: Testosterone: 17β-hydroxy-4-androsten-3-one; 4-androstenedione: 4-androstene-3,17-dione; dehydroisoandrosterone: 3β-hydroxy-5-androsten-17-one; pregnenolone: 3β-hydroxy-5-pregnen-20-one; cyproterone acetate: 6-chloro-12α-methylene-17α-acetoxy-progesterone.

After centrifugation, 0·5 ml of the supernatant was transferred to a counting vial and 10 ml of Bray's solution was added. Radioactivity was measured in a liquid scintillation counter (Packard Model 3300).

With this technique, the blank values, systematically controlled, do not differ significantly from the zero value of the standard curve: a maximum of only 0·10–0·20 ng per 2 ml of water blank was found in a few experiments.

In normal subjects the plasma testosterone levels were $0{\cdot}620 \pm 0{\cdot}160$ μg/100 ml in males and $0{\cdot}062 \pm 0{\cdot}022$ μg in females; the 4-androstenedione values were $0{\cdot}074 \pm 0{\cdot}037$ μg in males and $0{\cdot}151 \pm 0{\cdot}056$ μg in females.

Urinary 17-ketosteroids were estimated by the method of Drekter *et al*[11], and 17-hydroxycorticosteroids by the method of Silber and Porter[12].

RESULTS

Studies were carried out on twenty patients aged between 57 and 74, affected by prostatic carcinoma IV Flocks[13]. The first group of 8 cases had never received any form of hormonal treatment, the second group of 6 cases (9–14) had been treated with diethylstilbestrol (250 mg intravenously daily for 20 days) and the third group of 6 cases (15–20) and received oral doses of cyproterone acetate (200 mg daily for 20 days).

All these patients underwent bilateral orchiectomy, and the spermatic and peripheral venous blood samples were obtained simultaneously at the time of the operation (9–10 a.m.). The pre-treatment blood samples had also been taken at 9–10 a.m.

The mean peripheral plasma testosterone concentration was evaluated before the treatment in 19 patients affected by prostatic adenocarcinoma: the values were $0{\cdot}446 \pm 0{\cdot}157$ μg/100 ml with a range of 0·240–0·854 μg/100 ml; these results, which are slightly below the mean values from normal men aged between 18 and 40 yr ($0{\cdot}620 \pm 0{\cdot}160$, range of 0·406–0·875 μg/100 ml), were still within the normal range with the exception of cases 2 and 13 in whom plasma testosterone was decreased with values of 0·240 and 0·277 μg/100 ml respectively. Also normal were the urinary 17-ketosteroids and 17-hydroxycorticosteroids.

In the same 19 patients the mean peripheral plasma concentration of 4-androstenedione was increased ($0{\cdot}169 \pm 0{\cdot}088$ μg/100 ml, range of 0·071–0·350 μg/100 ml) with respect to normal values ($0{\cdot}074 \pm 0{\cdot}037$, range of 0·020–0·130 μg/100 ml). It was interesting to note that the patients with the lowest plasma testosterone levels (cases 2 and 13) presented the highest values of 4-androstenedione in blood.

In the group of patients never treated with steroid hormones (cases 1–8), the spermatic venous effluent values for testosterone were $9{\cdot}792 \pm 5{\cdot}046$ μg/100 ml (range of 4·040–18·950 μg/100 ml), twenty times those of the respective peripheral concentration ($0{\cdot}428 \pm 0{\cdot}148$, range of 0·240–0·720 μg/100 ml). The lowest levels of testosterone in the spermatic vein were found in case 2 (74 yr)(4·040 μg/100 ml), who also showed a decreased peripheral concentration of the androgen (0·240 μg/100 ml)(Table 1).

In the same cases, spermatic venous effluent values for 4-androstenedione were $0{\cdot}854 \pm 0{\cdot}296$ μg/100 ml (range of 0·538–1·360 μg/100 ml), five times those of the respective peripheral circulation ($0{\cdot}189 \pm 0{\cdot}105$ μg/100 ml).

In the second group of patients (cases 9–14) the normal peripheral testosterone levels of $0{\cdot}460 \pm 0{\cdot}201$ μg/100 ml before treatment, decreased signifi-

Table 1. Peripheral (P) and spermatic (S) venous plasma concentrations of testosterone (T) and 4-androstenedione (A), urinary 17-ketosteroids (17 KS) and 17-hydroxycorticosteroids (17 OHCS) in patients with prostatic adenocarcinoma

Case	Age (yr)		T (μg/100 ml)	A (μg/100 ml)	17 KS (mg/24 h)	17 OHCS (mg/24 h)
1 M.F.	68	P	0·352	0·308	12·8	2·2
		S	6·005	1·027		
2 R.S.	74	P	0·240	0·350	14·6	2·4
		S	4·040	1·360		
3 M.G.	71	P	0·378	0·225	14·9	3·6
		S	7·233	0·727		
4 F.F.	59	P	0·384	0·175	16·2	2·2
		S	7·960	0·840		
5 G.R.	71	P	0·511	0·084	9·8	2·9
		S	12·972	0·538		
6 O.A.	67	P	0·720	0·085	12·6	2·8
		S	18·950	0·615		
7 F.U.	69	P	0·330	0·220	16·4	2·7
		S	7·004	1·141		
8 C.R.	65	P	0·515	0·071	10·8	3·1
		S	14·175	0·585		
Mean values		P	0·428 ± 0·148	0·189 ± 0·105		
Mean values		S	9·792 ± 5·046	0·854 ± 0·296		

cantly to μg 0·128 ± 0·070 μg/100 ml after diethylstilbestrol treatment ($P < 0·05$). The concentration of testosterone in spermatic vein blood was proportionally decreased with mean values of 0·915 ± 0·766 μg/100 ml (range of 0·173–2·350 μg/100 ml); comparing these results with those obtained in the first group of patients (cases 1–8) it can be seen that this difference was statistically significant at $P < 0·01$ (Table 2).

On the other hand the changes in 4-androstenedione concentration after diethylstilbestrol in the peripheral circulation of the same patients were not statistically significant; in fact this androgen, which is normal before treatment (0·158 ± 0·086 μg/100 ml), seems to rise slightly after estrogen therapy (0·177 ± 0·116 μg/100 ml, range of 0·081–0·394 μg/100 ml), with no significant difference at $P > 0·05$. The slight decrease of 4-androstenedione in the spermatic vein blood after diethylstilbestrol administration is also of no statistical significance (0·711 ± 0·349 μg/100 ml, range of 0·364–1·210 μg/100 ml), when these values are compared with those obtained in the first group of patients: $P > 0·05$.

Table 3 shows the results obtained in the third group of patients treated with cyproterone acetate (cases 15–20). Mean peripheral plasma concentration of testosterone before treatment was 0·457 ± 0·143 μg/100 ml and decreased to levels of 0·177 ± 0·079 μg/100 ml (range of 0·070–0·300 μg/100 ml) after treatment ($P < 0·05$). The mean spermatic vein effluent levels of testosterone were also decreased after cyproterone acetate (1·800 ± 1·064 μg/100 ml, range of 0·657–3·633 μg/100 ml) and when compared with the mean values of the first group of patients (cases 1–8) the fall was statistically significant at $P < 0·01$.

The behaviour of 4-androstenedione after cyproterone acetate did not differ significantly from that observed after diethylstilbestrol; in fact the pre-treatment

Table 2. Effects of estrogen (diethylstilbestrol) therapy on peripheral (P) and spermatic (S) venous plasma concentrations of testosterone (T) and 4-androstenedione (A) in patients with prostatic adenocarcinoma

Case	Age (yr)	Before treatment 17 KS (mg/24 h)	17 OHCS (mg/24 h)		Before treatment T (μg/100 ml)	A (μg/100 ml)	After estrogens T (μg/100 ml)	A (μg/100 ml)
9 Fo.L.	72	13·1	2·6	P	0·415	0·096	0·230	0·081
				S			2·350	0·483
10. L.L.	57	11·4	2·2	P	0·455	0·082	0·134	0·108
				S			0·688	0·621
11 L.S.	67			P	0·381	0·190	0·183	0·096
				S			1·126	0·364
12 P.S.	68	9·2	3·2	P	0·380	0·172	0·098	0·195
				S			0·556	1·085
13 Fi.L.	66	8·8	2·6	P	0·277	0·310	0·033	0·394
				S			0·173	0·505
	61	12·4	1·8	P	0·854	0·101	0·091	0·189
				S			0·600	1·210
Mean values				P	0·460 ± 0·201	0·158 ± 0·086	0·128 ± 0·070	0·177 ± 0·116
				S			0·915 ± 0·766	0·711 ± 0·349

Table 3. Effects of cyproterone acetate therapy on peripheral (P) and spermatic (S) venous plasma concentrations of testosterone (T) and 4-androstenedione (A) in patients with prostatic adenocarcinoma

Case	Age (yr)	Before treatment 17 KS (mg/24 h)	17 OHCS (mg/24 h)		Before treatment T (μg/100 ml)	A (μg/100 ml)	After cyproterone acetate T (μg/100 ml)	A (μg/100 ml)
15 U.G.	65	9·2	2·2	P	0·315	0·248	0·200	0·307
				S			2·200	1·066
16 C.O.	69	13·5	4	P	0·689	0·071	0·123	0·110
				S			0·990	0·677
17 N.D.	72	9·8	3·3	P	0·410	0·170	0·300	0·134
				S			3·633	0·281
18 C.S.	52	11·6	3·4	P	0·487	0·100	0·162	0·121
				S			1·350	0·705
19 D.D.	66	14·8	2·4	P			0·212	0·120
				S			1·954	0·490
20 D.N.	62	14·6	2·8	P	0·387	0·168	0·070	0·205
				S			0·675	0·951
Mean values				P		0·151 ± 0·069	0·177 ± 0·079	0·166 ± 0·077
Mean values				S		0·457 ± 0·143	1·800 ± 1·064	0·695 ± 0·288

levels of this androgen in the peripheral circulation ($0{\cdot}151 \pm 0{\cdot}069$ μg/100 ml) seemed to increase slightly after treatment to μg $0{\cdot}166 \pm 0{\cdot}077$ μg/100 ml (range of 0·110–0·307 μg/100 ml), but this rise is not significant ($P > 0{\cdot}05$).

Similar results were obtained in the spermatic vein blood: the values of $0{\cdot}695 \pm 0{\cdot}288$ μg/100 ml (range of 0·281–1·066 μg/100 ml) after cyproterone acetate, compared with those of $0{\cdot}854 \pm 0{\cdot}296$ μg/100 ml of 4-androstenedione in the first group of untreated patients, seem to be decreased but the variation is not significant at $P > 0{\cdot}05$.

DISCUSSION

The evaluation of the androgenic activity in man is difficult on account of the complex androgen metabolism: in fact metabolites such as testosterone and 4-androstenedione are secreted not only by the gonads but also by the adrenal glands; in addition they are formed peripherally in the extraglandular compartments from other steroid precursors and then interconverted.

At present, the most direct evidence of testicular androgen secretion is the evaluation of testosterone and 4-androstenedione concentration in blood, collected simultaneously from the spermatic vein and the peripheral circulation.

Almost all our patients affected by prostatic adenocarcinoma IV Flocks, aged between 57 and 74 years, presented a normal androgenic status, with a mean value of testosterone in peripheral blood of $0{\cdot}446 \pm 0{\cdot}157$ μg/100 ml, which is similar to that reported by us in younger normal men and by Gandy and Peterson[14] in the same pathological conditions. This finding also confirms the results of Coppage and Cooner[15], and Kent and Acone[16] who failed to demonstrate significant differences between plasma testosterone levels in normal subjects, whether under or over 60 yr of age.

Testosterone was also isolated in spermatic venous blood of our patients before treatment and the values obtained were $9{\cdot}792 \pm 5{\cdot}046$ μg/100 ml, comparable to those found by Gandy and Peterson[14] in cases of adenocarcinoma and benign hypertrophy of the prostate (2·3–31·1 μg/100 ml) and by Hollander and Hollander[17] in normal men (4–10 μg/100 ml); they are, however, lower than the normal values reported by Hudson *et al.*[18], Dupré *et al.*[19] and Jeffcoate *et al.*[20].

Therefore these results clearly demonstrate that in patients with prostatic adenocarcinoma, the gonads still maintain normal levels of testosterone in blood.

The peripheral plasma levels of 4-androstenedione appear, in agreement with Gandy and Peterson[14], to be higher than those found in normal males, with values of $0{\cdot}169 \pm 0{\cdot}088$ μg/100 ml. Comparatively the spermatic venous plasma levels for this compound were $0{\cdot}854 \pm 0{\cdot}296$ μg/100 ml, which are similar to those obtained by Gandy and Peterson[14] in the same pathological conditions (0·720–1·550 μg/100 ml). At present we have not been able to establish the origin of these higher peripheral concentrations of the steroid.

The effect of high doses of estrogens on the plasma concentration of these two androgens was assayed in 6 cases aged between 57 and 72 yr. In peripheral circulation testosterone, which was normal before treatment, decreased significantly after diethylstilbestrol with a $P < 0{\cdot}05$. This drop followed a decreased secretion of testosterone from the testis, as is shown by the low values of the steroid in spermatic vein when compared to those of the first group of untreated patients ($P < 0{\cdot}01$).

Estrogen treatment did not, however, significantly affect the levels of 4-androstenedione in peripheral and spermatic venous blood; in fact the values for this metabolite in the cubital vein were $0{\cdot}177 \pm 0{\cdot}116\ \mu g/100$ ml, which are not significantly higher than the respective pre-treatment levels, whilst in the spermatic vein the levels were $0{\cdot}711 \pm 0{\cdot}349\ \mu g/100$ ml and these are in fact fairly comparable to those obtained in the untreated patients.

Therefore it would appear from our experiments that estrogens do not affect the testicular secretion of 4-androstenedione, at least not in the limits of our experimental conditions; further studies on a larger number of cases are needed to ascertain whether or not the increase of 4-androstenedione in peripheral plasma and the decrease in the spermatic vein is statistically significant, in which case the higher peripheral values of the androgen can be attributed to an extragonadal origin.

Treatment with cyproterone acetate of cases 15–20, aged between 52 and 72 yr, decreased the peripheral venous concentration of testosterone, as observed after diethylstilbestrol ($P < 0{\cdot}05$) These results are in agreement with the findings of Geller *et al.*[5] in patients with prostatic adenocarcinoma and by Sorcini *et al.* [21] in normal subjects. Testosterone in the spermatic vein was comparatively lower than that found in untreated patients ($P < 0{\cdot}01$).

The variations of 4-androstenedione levels in peripheral and spermatic vein blood after cyproterone acetate have no statistical significance, the behaviour being comparable to that observed after diethylstilbestrol; as in the case of estrogen therapy, the same considerations can be made.

In conclusion this compound appears to express its antiandrogenic effect mainly on the testicular function, inhibiting the secretion of testosterone, which is one of the most important hormonal factors in the growth of prostatic adenocarcinoma. Consequently the volume of the tumour may decrease, as a result of the lower uptake of testosterone by the neoplastic tissue.

These data *in vivo* therefore confirm, strictly from a quantitative point of view, our *in vitro* findings which demonstrated the inhibitory action of diethylstilbestrol and cyproterone acetate on the biosynthesis of testosterone from [4-^{14}C] pregnenolone, and are in agreement with the histological findings. In fact, in patients treated with estrogens de la Balze *et al.*[22] reported the disappearance of adult Leydig cells and the appearance of fibroblastic-like cells, and Markewitz *et al.* [23], after cyproterone acetate, observed edema of the interstitial connective tissue and damage of Leydig cells with cytoplasmic vacuolization, nuclear membrane shrinkage or pyknosis and cell pigmentation.

Nevertheless the exact intimate mechanism of this antiandrogenic action is not yet fully understood and further research is required to elucidate this problem.

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