Urological Research 6, 95-102 (1978)

Urological Research

Blood Coagulation Studies in Patients with Advanced Carcinoma of the Prostate Treated with 2,6-Cis-Diphenylhexamethylcyclotetrasiloxane or Estramustine-17-Phosphate

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Accepted: December 7, 1977

Summary. Two drugs, 2,6-cis-diphenylhexamethylcyclotetrasiloxane (Cisobitan) and estramustine-17-phosphate (Estracyt) were given to patients with poorly differentiated metastatic carcinoma of the prostate. The effect of the drugs on blood coagulation was investigated. Some parameters showed changes during the treatment: Antithrombin III decreased in the Estracyt treated patients to a level which might imply a thrombogenic effect. Fibrinogen decreased, whereas factor VIII showed no consistent change. Normotest changes appeared to correlate with liver damage whereas antithrombin III showed no change. Increased levels of fibrinogen degradation products and fibrinopeptide A (FPA) were more frequent in the group of deteriorating patients. However, the number of FPA analyses were too small for any definite conclusions regarding possible disseminated intravascular coagulation.

Key words: Coagulation - Malignancy - Prostate - Drugs - Oestrogen - Antithrombin.

A new compound 2,6-cis-diphenylhexamethylcyclotetrasiloxane (Cisobitan) with specific oestrogen-like effects on the male genital organs has been used for the treatment of patients with poorly differentiated metastatic prostatic carcinoma. Clinical results as well as some laboratory studies comparing this drug with estramustine-17-phosphate (Estracyt) have been reported by Edsmyr et al. (8). Conventional oestrogen treatment has frequently been associated with thrombosis. The present study attempts to evaluate whether these drugs have a primary effect on blood coagulation. Clotting factors are also compared in patients with differing clinical courses.

MATERIALS AND METHODS

Case Series

Twenty-seven patients were originally examined (mean age 67 years, range 53-81 years). All had poorly differentiated prostatic carcinoma, verified cytologically by transrectal aspiration biopsy and with radiologically verified skeletal metastases. The patients were evaluated by repeated clinical examinations and blood coagulation analyses. Samples for coagulation analyses were collected from Nov. 1973 through May 1975. They were obtained before treatment started and up to 4 months thereafter. However, the later samples could not always be obtained, mostly because of the poor state of the patients.

Thus from 9 of the 27 patients only one sample before the institution of the drug was

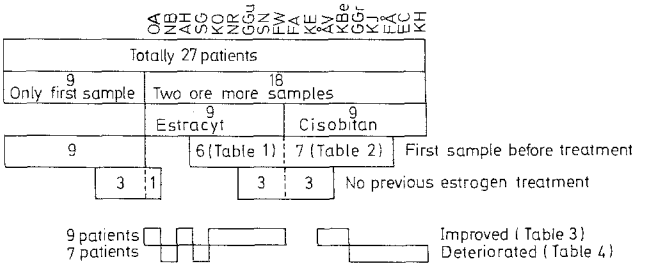

Fig. 1. Case series

0300-5623/78/0006/0095/$01.60

Table 1. Blood coagulation analyses in 6 patients before and after one month of treatment with Estracyt. Three had received oestrogen prior to study

Analysis		Patient						Normal range
		No oestrogen			Oestrogen			
		GGu	SN	FW	SG	KO	NR	
Prothrombin % of normal	a)	113	118	98	105	104	113	70-130
	b)	64	98	55	95	97	110	
Fibrinogen g/l	a)	2.6	2.7	2.7	3.5	4.0	3.5	1.8-3.0
	b)	2.8	2.7	2.8	3.9	2.7	3.4	
FDP mg/l	a)	<10	<10	<10	>40	<10	<10	<10
	b)	<10	<10	-	10-40	<10	<10	
FPA µg/l	a)	<0.9	-	0.9	1.1	<0.9	6.0	<2.0
	b)	<0.9	<0.9	-	1.3	3.1	<0.9	
Antithrombin III % of normal	a)	92	94	96	93	78	96	81-119
	b)	76	62	70	87	85	102	
α_2-macroglobulin % of normal	a)	115	92	105	114	99	75	55-145
	b)	118	72	135	134	108	86	

a) before start of treatment, b) after one month of treatment

obtained. Three of these patients had not received any previous oestrogen treatment (Fig. 1).

Eighteen patients were followed clinically and with coagulation analyses for a minimum of one, usually two months treatment with either drug. Nine were treated with 2,6-cis-diphenylhexamethylcyclotetrasiloxane (Cisobitan, AB Kabi) 100 mg three times daily and 9 with estramustine-17-phosphate (Estracyt, Leo AB) 300 mg twice daily. Five of the 18 patients had already been treated with the drug for a few days at the time of the first sampling. From 13 of the 18 patients the first sample was obtained before or within one day of starting the new drug. Six of these 13 had not received any oestrogen therapy and the other 7 had a free period of at least one month before institution of the new drug (Fig. 1).

Laboratory Methods

Coagulation Analyses: Samples were obtained by venepuncture with a sharp 1.4 mm gauge needle. After the first few ml of blood had been discarded, the blood was allowed to run directly into tubes containing an anticoagulant or antifibrinolytic reagent and immediately mixed thoroughly with the reagent.

Bleeding time was determined by the Ivy method (24). A platelet count was determined (19) and activated partial thromboplastin time (APTT) obtained with reagents from General Diagnostics using an incubation time of 6 m (27, 30). Normotest and Thrombotest analyses were performed using reagents from Nyegaard, Oslo. Prothrombin was determined specifically in a two-stage system using freeze-dried reagents from Imco, Stockholm (25).

Factor VIII activity was assayed in plasma samples in a one-stage system measuring the effect of the patient's plasma on the prolonged recalcification time of factor VIII deficient plasma (22).

Factor IX activity was assayed in a one-stage system similar to that of factor VIII but using factor IX deficient plasma (23).

Fibrinogen determinations were made with a polymerisation test using capillary blood (33). Fibrinogen degradation products (FDP) were determined by using Wellcome reagent

Table 2. Blood coagulation analyses in 7 patients before and after one month of treatment with Cisobitan. Four had received oestrogen prior to study

Analysis		Patient							Normal range
		No oestrogen			Oestrogen				
		FA	KE	ÅV	KBe	GGr	KJ	FÅ	
Prothrombin	a)	-	169	106	144	79	95	95	70-130
% of normal	b)	92	132	79	133	134	102	117	
Fibrinogen	a)	4.6	2.7	>6.0	5.4	2.6	4.6	4.0	1.8-3.0
g/l	b)	3.8	2.9	3.4	4.1	2.4	3.8	4.4	
FDP	a)	>40	10-40	10-40	10-40	40-80	<10	10-40	<10
mg/l	b)	>40	<10	10-40	<10	<10	<10	40-80	
FPA	a)	2.9	<0.9	1.6	-	-	-	5.6	<2.0
µg/l	b)	1.4	<0.9	<0.9	-	-	0.9	4.1	
Antithrom-	a)	150	141	110	109	71	85	84	81-119
bin III	b)	100	119	98	103	79	84	92	
% of normal									
α_2-macro-	a)	65	80	80	87	68	108	77	55-145
blobulin	b)	92	95	109	94	65	124	111	
% of normal									

a) before start of treatment, b) after one month of treatment

(29) and fibrin monomers were assayed by the ethanol gelation test (17).

Antithrombin III, plasminogen and α_2-macroglobulin were determined by a quantitative radialimmunodiffusion technique (10, 20).

Fibrinopeptide A was assayed by the radioimmunoassay technique of Nossel et al. (26) as described by Kockum (18).

Pooled plasma from 20 normal persons, aged 20-30 years was used as a reference for the assays of factors VIII and IX, antithrombin III, plasminogen and α_2-macroglobulin.

Normal ranges were obtained from 20 normal persons aged 21-60 years and expressed as mean $\pm$ 2 SD.

RESULTS

Investigation Prior to Treatment

Twenty-two patients were examined prior to or within one day of start of treatment. Thirteen had previously had conventional oestrogen therapy and 9 had had no previous hormone treatment. One patient had received radiation therapy. Most of them were in poor general condition.

The patients usually had high levels of factor VIII (15 / 22 >140 %; 6 / 22 >220 %) and fibrinogen (15 / 22 >3.1 g/l and another 5 above normal mean value) and often an increased platelet count (6 / 22 >360 x 10^9/l; 4 / 22 >440 x 10^9/l). Many had a raised level of fibrinogen degradation products (FDP) (12 / 22 >10 mg/l; 5 / 22 >40 mg/l) and 3 of these had a positive ethanol gelation test, indicating the presence of fibrin monomers.

Fibrinopeptide A (FPA) levels were high in 10 of the 18 patients investigated.

Normotest (NT) was either normal or high (5 patients) but in 7 of the patients Thrombotest (TT) values were low or borderline (23-40 %) and in 6 of the latter the NT/TT discrepancy $\frac{(NT\,\% - TT\,\%)}{NT\,\%}$ was ≥ 0.4 (32).

In at least 5 patients there was probably low grade intravascular coagulation with raised FDP levels, a high NT/TT ratio, a positive test for fibrin monomers or elevated

Table 3. Blood coagulation analyses in 9 patients with clinical signs of improvement, of whom four had received oestrogen prior to study

		No oestrogen					Oestrogen				Normal range
Analysis		OA	GGu	SN	FW	ÅV	KBe	AH	KO	NR	
Normotest	a)	110	96	85	90	140	160	140	92	180	70-130
% of normal	b)	110	130	115	130	140	140	130	120	110	
Prothrombin	a)	98	113	118	98	106	144	161	104	113	70-130
% of normal	b)	105	119	-	87	113	108	125	99	98	
Factor VIII	a)	158	98	120	160	193	320	163	248	163	65-135
% of normal	b)	102	213	170	145	120	210	195	139	101	
Fibrinogen	a)	4.5	2.6	2.7	2.7	6.0	5.4	2.8	4.0	3.5	1.8-3.0
g/l	b)	3.2	2.4	2.5	3.9	3.3	3.7	2.8	2.8	3.1	
FDP	a)	10-40	<10	<10	<10	10-40	10-40	<10	<10	<10	<10
mg/l	b)	<10	<10	<10	<10	<10	<10	<10	<10	<10	
FPA	a)	1.1	<0.9	3.5	<0.9	1.9	-	-	<0.9	6.0	<2.0
µg/l	b)	2.9	<0.9	0.9	2.0	<0.9	<0.9	-	<0.9	-	
Antithrombin III	a)	103	92	94	96	110	109	85	78	96	81-119
% of normal	b)	70	79	72	77	94	101	98	75	97	
Drug	c)	E	E	E	E	C	C	E	E	E	
Observation period months		3	3	2	4	3	3	4	3	3	

a) at start of observation period
b) at end of observation period
c) C = Cisobitan, E = Estracyt

FPA values. In 4 of the 5 patients 3 of these parameters were pathological. The fifth patient who was treated with Warfarin showed increased levels of FDP and FPA.

Patients previously treated with oestrogen appeared to differ from untreated ones in the antithrombin III (AT III) level, although they had been without oestrogen for a minimum of one month. Thus, 3 out of 13 showed clearly subnormal values of 52-71% (normal 81-119%), and another 3 had borderline values, 78-85%. The mean value was 92% and median value 93%. In the 9 patients not previously treated with oestrogen the lowest value was 92%, mean value 110% and median value 104%.

Factor IX and plasminogen values, APTT and bleeding times were essentially normal.

Effect of Estracyt and Cisobitan

Samples were obtained before and one month after treatment from 6 patients in the Estracyt group and 7 in the Cisobitan group.

In the Estracyt group (Table 1) 3 of the 6 patients showed an AT III decrease to subnormal values after one month of treatment. These were the patients who had not received oestrogen previously. In the remaining patients the AT III values were unchanged. Some decrease was also noted in the prothrombin values in those patients not previously treated with oestrogen. However, these later became normal (cf. Tables 3 and 4). There was a minimal increase in α_2-macroglobulin and no change in fibrinogen values.

In the Cisobitan group 3 patients had not received any prior oestrogen treatment. In these there was a decrease in the AT III level, although no subnormal values were observed (Table 2). The percentage decrease was less than in the Estracyt group but the initial values were higher. There was some decrease in fibrinogen values and some increase in α_2-macroglobulin after one month's treatment.

No definite changes in bleeding time, APTT, platelet count, Normotest, factor VIII,

Table 4. Blood coagulation analyses in 7 patients with clinical signs of deterioration, all of whom had received oestrogen prior to study

Analysis		Patient							Normal range
		NB	EC	GGr	SG	KH	KJ	FÅ	
Normotest	a)	160	150	170	75	150	110	-	70-130
% of normal	b)	130	85	160	58	120	115	-	
Prothrombin	a)	90	117	79	105	113	95	95	70-130
% of normal	b)	121	74	154	74	128	84	117	
Factor VIII	a)	232	103	106	140	188	153	118	65-135
% of normal	b)	89	335	178	233	123	225	135	
Fibrinogen	a)	4.7	3.5	2.6	3.5	3.7	4.6	4.0	1.8-3.0
g/l	b)	4.3	2.3	2.8	2.9	3.2	4.1	4.4	
FDP	a)	<10	<10	40-80	>40	<10	<10	10-40	<10
mg/l	b)	<10	<10	10-40	>40	<10	10-40	>40	
FPA	a)	-	-	-	1.1	-	<0.9*	5.6	<2.0
µg/l	b)	1.5	-	-	2.2	<0.9	2.9	4.1	
Antithrom-	a)	73	80	71	93	107	85	84	81-119
bin III	b)	67	78	92	90	91	72	92	
% of normal									
Drug	c)	E	C	C	E	C	C	C	
Observation period, months		2	3	3	2	2	2	1	

a) at start of observation period
b) at end of observation period
c) C = Cisobitan, E = Estracyt
* after 1 month

factor IX or plasminogen values were seen in any of the groups.

Relationship of Changes in Blood Coagulation Factors to the Clinical Course

Sixteen of the 18 patients showed either subjective and objective signs of improvement during the observation period (improved appetite, weight-gain, less micturition difficulties, hormonal effect demonstrated by needle biopsy and/or a fall in acid phosphatases) or clearly deteriorated (Tables 3 and 4).

In 9 patients with objective and subjective signs of improvement, some return towards normal of raised levels of FDP was observed. In the patients who had not received any prior oestrogen treatment a decrease in AT III levels but no uniform change in factor VIII was seen after the observation period of 1-4 months. In improving patients with prior oestrogen treatment there was some decrease in factor VIII and no AT III change. Fibrinogen values were somewhat lowered.

In 7 patients with signs of deterioration, factor VIII tended to increase despite previous oestrogen treatment. More patients in this group showed elevated FDP and FPA values at the end of the observation period than in the group of patients who had improved. There was no significant change in AT III levels. Some subnormal values were noted but as in group 1 without any apparent correlation with low Normotest values.

We have deliberately refrained from applying tests of significance to these small numbers of heterogeneous data.

DISCUSSION

Patients with malignant diseases often have abnormalities in coagulation parameters such as high values of factor VIII and fibrinogen as well as signs of fibrinolysis (4, 5, 12, 14).

Metastasising prostatic carcinoma is often associated with acute disseminated intravascular coagulation (DIC), often with bleeding (11). A chronic form of intravascular coagulation has also been demonstrated. This is characterised by normal or even high levels of platelets and coagulation factors, due to increased synthesis, and raised levels of fibrinogen degradation products (FDP) and fibrin monomers (7).

DIC is mainly seen when the malignant disease is advanced. It is reasonable to assume that laboratory signs of DIC will be more pronounced in the advanced stage. Such analyses might even be of prognostic value. As can be seen in Table IV the deteriorating patients showed FDP and FPA increases at the second investigation. There is some increase in factor VIII activity which may also be associated with a hypercoagulable state. However, the decrease in Normotest values in our patients seems to be related mainly to liver damage and not to the isolated consumption of prothrombin as in DIC. The two cases with the lowest Normotest values had signs of liver metastases, which in one of them were confirmed at autopsy.

Though both of the compounds investigated have oestrogen-like properties we could not demonstrate much change in e. g. fibrinogen, factor VIII and factor IX which could be regarded as an oestrogen effect. However, in the patients who had not been treated with oestrogen previously, there was a decrease in AT III levels after administration of Estracyt and a similar but less pronounced decrease with Cisobitan. In most of the patients who had earlier been treated with oestrogen, the antithrombin level was already decreased. Low levels of AT III have been associated with increased intravascular coagulation and thromboembolic episodes. The best examples of a link between a low antithrombin level and thromboembolism are found in patients with hereditary deficiency (3, 9). Decreased AT III levels are also found in patients with severe liver disease and in DIC (1, 13). It has also been demonstrated post partum and in women taking oral contraceptives. In these cases it is associated with an increased oestrogen level (2, 16). Since malignancy as such implies an increased risk of thromboembolism the decrease in AT III probably enhances this risk, at least when values decrease to subnormal levels. Apart from the decrease in AT III levels the coagulation parameter changes in patients who had not received oestrogen and showed improvement imply reduced hypercoagulation.

Oestrogen lowers the fibrinolytic activity of the vein wall when administered to patients with prostatic carcinoma (6), although spontaneous fibrinolytic activity in peripheral blood and the fibrinolytic response to venous occlusion are not affected. In patients with prostatic carcinoma treated with Cisobitan the fibrinolytic system has been investigated by Isacson (15). Using the same dose of the drug as in this study no depression of fibrinolytic activity could be observed either in the vein wall or in peripheral blood before or after venous occlusion.

The reduction in fibrinogen values observed, especially in the patients on Cisobitan therapy, is of the same magnitude as that observed by Isacson (15) and seen during oestrogen treatment (31). Since oestrogen tends to increase fibrinogen values in normal persons, this reduction might be related to some amelioration of the malignant disease.

The small increase in α_2-macroglobulin, seen especially after one month's treatment with Cisobitan correlates well with the findings of Isacson (15) and is in accordance with the effects of oestrogen treatment. The α_2-macroglobulin values after a longer treatment period did not differ from pretreatment values in either of the two groups.

Most of the recorded changes of the coagulation parameters are relatively small and difficult to evaluate in this small case series. The marked decrease in AT III values, however, must be regarded as a clearly thrombogenic factor in a patient with a malignant tumour that in itself predisposes to thrombotic disease. The difference between the two drugs may at least partly be dose-related. Further studies on an extended and less heterogeneous case series would be of interest.

Acknowledgements. This work was supported by grants from the Swedish Medical Research Council (No. 19X-520). We sincerely thank Miss Ulla Wallinder for her valuable assistance and Dr. Bengt Strindberg, AB Kabi, for stimulating discussion.

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