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Prolonged Bleeding Time Due to Mitotane Therapy

Harm R. Haak, Kathelijne M.J. Caekebeke-Peerlinck, Arnoud P. van Seters and Ernest Briët

After finding prolonged bleeding times in 2 patients treated with mitotane, we prospectively studied 7 patients with adrenocortical cancer on mitotane therapy. Before and 1 and 2 or more weeks after starting mitotane we determined the platelet counts, bleeding times and global coagulation parameters. All patients had a normal bleeding time before treatment. In 6 cases the bleeding time became prolonged (245-555 s). 4 patients exhibited platelet aggregation responses compatible with an aspirin-like defect. It is concluded that mitotane may cause a clinically relevant defect of platelet function.
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INTRODUCTION

ADRENAL CORTICAL carcinoma is an uncommon tumour in man. Mitotane is the drug of choice for patients with inoperable, recurrent and metastatic disease [1]. In our hospital mitotane is given even after apparently radical surgery because of frequent recurrences and the assumption that cytotoxic therapy is more effective when the tumour load is low [2]. Mitotane therapy is continued for 2 years if resection has been judged to be complete or for 1 year after apparent disappearance of the tumour. Serum trough concentrations of mitotane exceeding 14 mg/l were associated with a response rate of 50%, whereas no therapeutic effect was seen in patients with levels lower than 10 mg/l [2]. We therefore aim to achieve serum trough levels above 14 mg/l (when possible 25 mg/l). The mechanism of action of mitotane

Table 1. Characteristics of 7 patients with adrenocortical carcinoma treated with mitotane

Patient	Sex	Age (yr)	Tumour response	Side-effects
1	M	59	Complete remission*	CNS;GI
2	F	39	Progression	CNS;GI
3	F	30	Complete remission	CNS;GI;myopathy
4	F	72	Progression	CNS;GI
5	F	18	Progression	CNS;GI
6	F	33	Not known†	CNS;GI
7	F	40	Progression	CNS;GI

CNS = Central nervous system; GI = gastrointestinal.
*Only steroid production was detectable after operation.
†No tumour detectable after surgery. Mitotane given prophylactically.

in adrenal cortical carcinoma is related to its interference with the cytochrome P-450 dependent steps of steroid synthesis in adrenocortical mitochondria [3]. Irreversible damage of the mitochondria of the adrenocortical cells due to mitotane leads to cell destruction, producing focal degeneration of the fascicular and reticular zones [4]. Mitotane has also been found to be a potent inducer of hepatic microsomal P-450 cytochromes [5]. Toxic side-effects of mitotane therapy include anorexia, nausea, vomiting and diarrhoea. Severe (reversible) CNS toxicity, such as ataxia, impairment of intelligence and psychosis is most prominent when serum levels exceed 20 mg/l. Less frequent side-effects are rash (14%), miscellaneous (5%) and ocular (4%), genitourinary (3%) or cardiovascular (3%) abnormalities [6]. Mild leucopenia is observed sporadically.

We found prolonged bleeding times in 2 patients who received mitotane in 1985 and 1987. 1 of them had subcutaneous haemorrhages and menorrhagia due to endometrial hyperplasia. Since platelet function disorders have not yet been reported in association with mitotane, we prospectively studied the effect of mitotane on the bleeding time in 7 consecutive patients with adrenal cortical carcinoma.

PATIENTS AND METHODS

All patients with adenocortical carcinoma treated with mitotane since 1987 were included in the study (Table 1). There were 6 women and 1 man (age 18–72 years). All patients underwent surgery for their adrenocortical cancer. Only in 1 case (no. 6) was tumour resection considered complete. In patient 1 steroid production was the only sign of residual tumour remnants after surgery. In the remaining cases (nos 2–5 and 7) complete surgical removal of the tumour had not been possible. The mitotane therapy was started with 4–8 g per day in four equal doses administered in chocolate. For maintenance therapy mitotane was given as tablets (Bristol–Meyers). Serum levels of mitotane measured by gas-liquid chromatography as described previously [7], were between 10 and 51 mg/l. In 2 patients (nos 1 and 3) a tumour response to mitotane was obtained; patient no. 6 was still in complete remission 12 months after starting mitotane. In addition to mitotane all patients received hydrocortisone, fludrocortisone acetate and, when necessary, metoclopramide and loperamide. No medication known to influence the bleeding time was given. Nor did the patients use (over-the-counter) non-steroidal anti-inflammatory agents.

The following parameters were determined before starting mitotane therapy and 1 week and 2 or more weeks after initiation of mitotane treatment: platelet count, activated partial thromboplastin time (aPTT, Cephotest, Oslo), prothrombin time (PT), thrombin clotting time, fibrinogen and fibrin(ogen) degradation products (Thrombo-Wellcotest, Wellcome, Dartford, England). The bleeding time was assessed according to Ivy [8] and when prolonged, platelet aggregation studies were carried out using a PAP-4 platelet aggregation profiler (Bio/data, Hatboro, USA) with adenosine diphosphate (ADP, Boehringer, Mannheim) at final concentrations of 0.2 μg/ml and 2 μg/ml, collagen (Horm, München) at a final concentration of 2.0 μg/ml, arachidonic acid (Bio/Data, Horsham, USA) at a final concentration of 0.5 mg/ml and ristocetin (Lundbeck, Copenhagen) at final concentrations of 0.6 mg/ml and 1.5 mg/ml. If the bleeding time was prolonged we also determined the level of von Willebrand factor antigen (vWF:Ag) by a Laurell technique [9] and the ristocetin cofactor activity (vWF:Rco) using lyophilised platelets (Bio/Data, Hatboro, USA) [10].

Blood for coagulation assays was collected in sodium citrate (109 mmol/l). Platelet-rich plasma was obtained by centrifugation for 15 min at 164 *g*, platelet-poor plasma was obtained by centrifugation for 15 min at 2500 *g*.

RESULTS

Before starting mitotane therapy, all 7 patients had a normal bleeding time and normal coagulation tests (Table 2).

After 1 week of treatment the bleeding times became prolonged in 6 cases (results not shown), with further prolongation in the course of continued treatment (Table 3). In all but 1 patient therapeutic levels of mitotane were reached. The exception was patient no. 5 who repeatedly had subtherapeutic levels (4 mg/l) due to poor drug compliance: the bleeding time returned to normal after initial prolongation. In all patients normal or high levels of vWF:Ag and vWF:RCo were found.

4 patients, each with a prolonged bleeding time, showed reduced platelet aggregation upon stimulation with ADP (2 μg/ml), or arachidonic acid. In 6 patients the aPTT was shortened. In one of these patients, who had a shortened PT as well, a slight degree of spontaneous aggregation was noted after stimulation with the subliminal dose of ADP (0.2 μg/ml). 2

Table 2. Bleeding times, platelet counts and coagulation tests before initiation of mitotane therapy

Patient	Bleeding time (s)	Platelet count (10^9/l)	aPTT (s)	PT (s)	Fibr. (g/l)	FDP
1	210	229	29.5	12.2	3.8	neg
2	120	330	24.0	12.1	2.6	neg
3	105	291	24.2	13.5	2.7	neg
4	135	249	22.0	12.3	3.8	neg
5	195	332	24.5	14.2	2.9	neg
6	150	277	20.0	9.9	3.8	neg
7	150	338	27.3	12.6	1.5	neg
Ref. values	60–240	150–400	28–36	11.5–14.5	1.7–3.7	neg

aPTT = activated partial thromboplastin time; PT = prothrombin time; fibr. = fibrinogen; FDP = fibrin(ogen) degradation products.

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Table 3. Laboratory data obtained during mitotane therapy

Patient	Weeks of treatment	Mitotane (mg/l)	Bleeding time (s)	Platelet count (10^9/l)	vWF:Ag (U/dl)	vWF:Rco (U/dl)
1	24	23	445	324	560	443
2	17	31	225	446	–	–
3	31	18	450	268	176	140
4	19	51	405	449	336	316
5	2	8	255	294	–	–
6	17	27	555	263	132	148
7	13	19	480	360	270	209
Ref. values			60–240	140–400	36–215	49–195

vWF:Ag = von Willebrand factor antigen, vWF:Rco = ristocetin cofactor activity.

patients exhibited a slightly increased aggregation after low-dose ristocetin (Table 4).

Platelet count, aPTT, PT, thrombin clotting time and fibrinogen remained normal during the study period.

DISCUSSION

O,p'DDD is the drug of choice for inoperable, recurrent or metastatic adrenal cortical carcinoma [1]. We also give the drug after apparently radical surgery in order to prevent the frequent recurrences. Our goal is to reach mitotane levels of at least 14 mg/l for long periods.

2 of our patients on mitotane therapy were found by chance to have long bleeding times. Since patients who are treated with mitotane are likely to undergo surgical procedures (e.g. diagnostic procedures when recurrence is suspected) and since one of our patients suffered clinical bleeding, we decided to evaluate the effect of mitotane on bleeding time prospectively.

In 6 of 7 consecutive patients we indeed found a prolongation of the bleeding time, detectable as early as 1 week after starting mitotane and with further prolongation in the course of continued administration. Only in patient (no. 5) did the bleeding time return to normal after initial prolongation; however, she also had a very low level of mitotane (4 mg/l) at that moment and did not take the drug regularly because of severe gastrointestinal and CNS toxicity.

The prolongation of the bleeding time was due to a platelet function defect since the platelet count remained constant throughout the study period and an acquired form of von Willebrand's disease was ruled out. For 5 of the 6 patients with a prolonged bleeding time we found abnormalities of platelet aggregation. Because of the irregular pattern of the abnormal results of platelet aggregation studies no definite conclusions about the underlying cause can be made. In the patient with subtherapeutic mitotane levels platelet aggregation was normal.

Since the effect of mitotane on adrenal cortical carcinoma has been attributed to toxic interference with cytochrome P-450 dependent steps of steroid synthesis [3,11], a possible explanation for the platelet function defect could be inhibition of thromboxane synthase. This enzyme, characterised as a cytochrome P-450 enzyme [12], plays an important role in the arachidonic acid pathway of platelets. Further research will be needed to elucidate the pathogenesis of this mitotane induced defect.

The clinical implications of the prolonged bleeding time are difficult to assess. Awareness of a prolonged bleeding time is presumably important when invasive or therapeutic procedures are required. Endometrial hyperplasia is often encountered during mitotane therapy. In our view this might be due to an oestrogen-like effect of mitotane. 1 of our patients with endometrial hyperplasia suffered vaginal bleeding almost continuously during mitotane treatment; we tried, without success, to correct the bleeding time with 1-deamino-8-D-arginin vasopressin (DDAVP). She later underwent a curettage without excessive blood loss; her bleeding time was 480 s at that time.

We conclude that mitotane in high doses gives rise to a moderate prolongation of the bleeding time. Although this seldom caused spontaneous bleeding in our patients, it may be clinically important when surgical intervention is considered.

Table 4. Response to agonists of platelet aggregation in the 6 patients with a prolonged bleeding time during mitotane therapy

Patient	ADP (μg/ml) 0.2	ADP (μg/ml) 2	Collagen	Arachidonic acid	Ristocetin (mg/ml) 0.6	Ristocetin (mg/ml) 1.5
1	–	±	±	±	–	+
3	–	±	+	+	–	+
4	–	±	+	±	±	+
5	–	+	+	+	–	+
6	±	+	+	±	±	+
7	–	±	+	±	–	+
Control	–	+	+	+	–	+

+ = maximal response, ± = weak or reversible response, – = no response.

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Overall Survival of Breast Cancer Patients in Relation to Preclinically Determined Total Serum Cholesterol, Body Mass Index, Height and Cigarette Smoking: a Population-based Study

Lars J. Vatten, Olav P. Foss and Stener Kvinnsland

Mean overall 5-year survival related to preclinically determined total serum cholesterol, body mass index (BMI), height and cigarette smoking has been analysed among 242 incident cases of breast cancer aged 36–63 years that developed in a population of 24 329 Norwegian women during a mean follow-up of 12 years (range 11–14). The study factors were ascertained at least 1 year prior to diagnosis (mean = 8 years), and the cases have been followed up with respect to death for a mean time of approximately 5 years after diagnosis. Patients whose preclinical total serum cholesterol values were within the highest quartile (≥7.52 mmol/l, mean = 8.58 mmol/l) of the underlying population had a hazard ratio of dying of 2.0 (95% confidence limits, 1.1 and 3.7) compared to cases with cholesterol values in the lowest quartile (mean = 5.28 mmol/l), after adjustment for age at diagnosis, clinical stage, and body mass index. In relation to BMI (Quetelet's index: weight/height^2) patients who were obese prior to diagnosis were at higher risk of dying than those who were lean. Compared to patients in the lowest quartile of BMI (mean Quetelet = 21), the hazard ratio was 2.1 (95% confidence limits, 1.2 and 3.8) for patients in the highest quartile (mean Quetelet = 30), after adjustment for age at diagnosis, clinical stage, and total serum cholesterol. For height and for cigarette smoking, no relation with survival was observed. A potential problem of this study might be insufficient information about other well known prognostic factors, but the results suggest that preclinical total serum cholesterol and BMI are positively associated with the risk of dying among women who develop breast cancer.
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INTRODUCTION

NO OVERALL association has been shown between total serum cholesterol and the risk of developing breast cancer in women [1, 2], but there is some evidence for an inverse relation between total serum cholesterol and risk of premenopausal breast cancer [2, 3]. Once disease is manifest, however, total serum cholesterol may have an adverse influence on the prognosis of breast cancer patients [4].

Body mass index (BMI) appears to be related to the risk of breast cancer, but in opposite directions, depending on the menopausal status of the women. Whereas a high body mass may increase breast cancer risk among postmenopausal women [5], some studies have indicated that BMI is negatively associated with the risk of developing breast cancer in premenopausal women [2, 6–8]. Among patients, however, it has been suggested that increased body mass has an adverse effect on survival [4, 9–12].

The evidence suggesting that height is positively associated with the risk of developing breast cancer [7, 13, 14], has been interpreted as an effect that might be related to nutritional influences during perimenarcheal age [15, 16], an important phase for height determination and for breast tissue development. With respect to prognosis, there is little support for any relation between height and survival in breast cancer patients [12].

Cigarette smoking is not likely to be related to breast cancer risk [17], despite hypotheses suggesting a possible negative association [18]. In relation to survival, it might be of interest to examine the hypothesis that cases who smoke have a better prognosis than cases who do not smoke, possibly due to anti-oestrogenic effect of smoking [19].

This study distinctly differs from most other studies of these aspects in two important respects. Firstly, we report mean 5-year overall survival among 242 cases of breast cancer that were accrued in a fixed cohort of 24 329 Norwegian women during approximately 12 years of follow-up, making this a population-based study. Secondly, survival has been analysed in relation to

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