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Expression of P-Glycoprotein in Relation to Clinical Manifestation, Treatment and Prognosis of Adrenocortical Cancer

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The presence of P-glycoprotein, associated with multiple drug resistance and present in the normal adrenal cortex, was studied in 15 cases of adrenocortical carcinoma. P-glycoprotein was found in eight tumours; no correlation was found with clinical presentation, steroid production or histological index. 10 patients received mitotane. Remarkably, 3 patients with P-glycoprotein-positive tumours achieved complete remission. On the other hand, 2 patients with P-glycoprotein-negative tumours showed progression of the disease despite mitotane treatment. These findings suggest that the expression of P-glycoprotein in adrenocortical carcinoma is not related to clinical manifestations, steroid production, histological index or response to mitotane therapy.
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INTRODUCTION

ADRENOCORTICAL CARCINOMA is resistant to most chemotherapeutic agents. Mitotane (o,p'-DDD) is the drug of choice for metastatic, or recurrent adrenocortical carcinoma [1]. The response rates reported for mitotane range from 29 to 61% [2, 3]. Serum levels exceeding 14 mg/l seem to correlate best with tumour response [4]. However, most patients will suffer a recurrence or develop metastases.

In a variety of tumours multiple drug resistance (MDR) is associated with expression of the MDR-1 gene, leading to the presence of P-glycoprotein. P-glycoprotein is a plasma membrane-bound transport protein that causes decreased (cytotoxic) drug accumulation within the cell [5, 6]. Since P-glycoprotein is expressed in the normal adrenal cortex [5, 7] it is possible that this effusion system is responsible for the intrinsic resistance of adrenocortical carcinoma to cytotoxic agents.

We investigated the relationship between the presence of P-glycoprotein, clinical manifestations, morphological parameters and treatment of adrenocortical cancer.

PATIENTS AND METHODS

Patients

15 adrenocortical carcinoma patients (12 female, 3 male) were enrolled in the study. All patients had undergone surgery. The operation was considered radical in only 4 of the 15 patients.

10 patients received mitotane therapy. Therapy was started at 4–8 g/day in four equal doses. Mitotane serum concentrations of over 14 mg/l, determined according to the method described by Moolenaar [8], were aimed at.

Evaluation of response was based on the standard criteria of complete remission (no detectable disease), partial response ($\geq$ 50% reduction), minimal response ($\geq$ 25–$<$ 50% reduction), stable disease and progressive disease ($\geq$ 25% increase).

Immunohistochemistry

Fresh-frozen tumour tissue (−75°C) was stained immunohistochemically with the murine monoclonal antibody JSB-1 [9] against P-glycoprotein, according to the immunoperoxidase amino-ethyl-carbazole (AEC) method [10]. The presence of P-glycoprotein was scored as − (negative), + (10–24% cells positive), ++ (25–49% cells positive), +++ (50–74% cells positive), and ++++ (75% or more cells positive). A normal adrenal gland served as a positive (cortex) as well as negative control (medulla).

The patients were divided in two groups: P-glycoprotein-negative (group 1) and P-glycoprotein-positive (group 2).

Histological index

Seven parameters (regressive changes, preservation of normal structure, nuclear atypia, nuclear hyperchromasia, structure of nucleoli, mitotic activity and invasion of the capsule and/or vascular wall), each with a specific discriminating value, determine the histological index according to van Slooten *et al.* [11]. The maximal index is 28.4. A tumour with an index above 8 is considered malignant.

Steroid production

Steroid production was assessed by determination of the pretreatment serum level of dehydroepiandrosterone sulphate and the excretion of 17-oxo and 17-oxogenic steroids in 24-h urine corrected for 10 mmol creatinine excretion.

Statistical analysis

The Mann–Whitney test was used for statistical analysis.

RESULTS

Expression of P-glycoprotein

P-glycoprotein was present in eight tumours (three ++++, two +++, two ++ and one +), and absent in seven tumours.

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Table 1. Steroid production, histological index and P-glycoprotein expression

	P-glycoprotein-negative (group 1) $n = 7$	P-glycoprotein-positive (group 2) $n = 8$	
Dehydroepiandrosterone sulphate (μmol/l)	7.9 ± 10	8.1 ± 11	NS
17-Oxo-steroids (μmol/24-h/10 mmol creatinine)	155 ± 191	97 ± 49	NS
17-Oxogenic-steroids (μmol/24-h/10 mmol creatinine)	68 ± 64	44 ± 28	NS
Histological index	21.4 ± 5.6	25.0 ± 4.3	NS

Values are expressed as mean ± S.D.

Clinical presentation and steroid production

Clinical hormonal activity was observed in 11 patients. The expression of P-glycoprotein was not related to the clinical symptoms or to steroid production (Table 1).

Histological index

The histological index of the tumours ranged from 13.6 to 28.4 in the 15 patients. In 3 patients with an index of 28.4 the tumour was P-glycoprotein-positive: 25–> 75% of the cells were positive. 1 patient with the maximum index showed no P-glycoprotein immunoreactivity. The other 6 patients without P-glycoprotein in the tumour had a histological index between 13.7 and 24.2. The mean histological index in group 1 did not differ statistically significantly from the mean histological index in group 2 (Table 1).

Response to mitotane therapy: P-glycoprotein-positive tumours ($n = 7$) (Table 2)

3 patients (numbers 5, 6 and 8) achieved a complete remission. A minimal tumour response was seen in patient number 10. 2 patients did not respond to mitotane therapy. The results obtained in patient number 4 could not be evaluated because mitotane had not been received adjuvantly; the patient is tumour-free 1 year after starting mitotane.

Table 2. P-glycoprotein expression and tumour response in patients treated with mitotane

Patient no.	P-glycoprotein presence	Duration of mitotane therapy (months)	Mitotane level (mg/l)	Response
1	–	2	<14	PD
2	–	4	<14	PD
3	–	9	>14	PR, 5 months
4	+	12	>14	ND
5	++	6	>14	CR, 2 months
6	+++	24	>14	CR, 60 months*
7	+++	5	>14	PD
8	++++	12	>14	CR, 12 months
9	++++	4	>14	PD
10	++++	3	>14	MR, 2 months

CR = Complete response, PR = partial response, MR = minimal response, PD = progressive disease, ND = not determinable, mitotane prophylactically.
*Ongoing remission.

Response to mitotane therapy: P-glycoprotein-negative tumours ($n = 3$) (Table 2)

Patient number 3 (mitotane serum level over 14 mg/l) had a partial response lasting 5 months. Patients number 1 and 2 had progressive disease despite mitotane (serum levels below 14 mg/l).

DISCUSSION

Large variations in expression of the MDR-1 gene, indicated by the presence of P-glycoprotein, were observed. The presence of P-glycoprotein in adrenocortical carcinoma was not correlated with the histological index. Apparently, less well differentiated adrenocortical carcinomas do not lose the ability to express P-glycoprotein.

In the adrenals, P-glycoprotein is expected to be related to transport of steroids out of cells [5]. If P-glycoprotein plays an important role in steroid excretion, we would expect patients with a tumour lacking this protein to exhibit hormonal inactivity. Patients with pronounced P-glycoprotein expression would show overt hormonal activity. Our results, however, offer no support for the hypothesis that P-glycoprotein plays an important role in hormone transport out of adrenocortical cells.

2 patients with a tumour lacking P-glycoprotein who received mitotane therapy had progressive disease despite this therapy. Unfortunately, mitotane therapy in these cases was not optimal since the serum levels were below 14 mg/l. In 3 patients with P-glycoprotein expression on mitotane therapy, complete remission was achieved. These results indicate that the response of adrenocortical cancer to mitotane is not related to the presence or absence of P-glycoprotein. In fact, it was recently found that mitotane reverses MDR in adrenocortical carcinoma *in vitro* [12]. In adrenocortical carcinoma, as well as adrenocortical adenoma or hyperplasia, mitotane was found in all assayed samples [13]. Mitotane can inhibit steroid synthesis in normal and pathological adrenal glands [2, 14]. In view of the presence of P-glycoprotein in healthy and pathological adrenal cortices, it may be concluded that mitotane is not pumped out of the cells by the P-glycoprotein transport system, at least not in substantial amounts.

Research on adrenocortical cancer is limited by the rarity of this tumour. The results in our group of patients suggest, however, that resistance of adrenocortical cancer to mitotane is not regulated via the P-glycoprotein transport system. The clinical presentation, steroid excretion and histological index or degree of differentiation of the tumour are not related to the presence of P-glycoprotein. We are inclined to conclude at this time that expression of the MDR-1 gene is probably not important for determining the prognosis of patients with adrenocortical cancer.

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Feature Articles

Treatment Options in Clinical Stage I Non-seminomatous Germ Cell Tumours of the Testis: A Wager on the Future? A Review

J.P. Droz and A.T. van Oosterom

INTRODUCTION

THE PROGNOSIS of metastatic germ cell tumours of the testis has dramatically improved with the introduction of cisplatin-based chemotherapy [1]. One of the most important refinements in the treatment of these tumours is the use of prognostic factors to indicate risk-adapted treatments. This is currently used in the treatment of advanced disease [1]. It is noteworthy that even in the past the cure rate of patients with tumours localised in the testis only (clinical stage 1) was high [2, 3]. The standard treatment of these patients was either retroperitoneal lymph node dissection (RPLND) or radiotherapy to the retroperitoneal region and cure rates as high as 80–90% have been reported [2]. However, the "wait and see" policy (surveillance) emerged in the early 1980s [4] when it was proven that low volume metastatic disease could be cured by chemotherapy in the majority of cases and more reliable investigations including chest and abdominal computed tomography (CT) scan, but especially serum alpha-fetoprotein (AFP) and human chorionic gonadotrophin (HCG) measurements made earlier detection possible. This policy then became popular. Unfortunately, no evaluation of both modalities has been undertaken in a randomised trial. We review here the status of the different treatment options in localised testis cancer.

We only consider in this review non-seminomatous germ cell tumours of the testis as defined both in the World Health Organization (WHO) classification [5] and the English classification [6]. Clinical stage I disease is defined according to the Union Internationale Contre le Cancer staging system [7] as a disease without visceral and retroperitoneal lymph node metastases after physical examination, imaging and biochemical tests.

CLINICAL AND PATHOLOGICAL STAGE I, CLARIFYING THE PROBLEM

Clinical staging is based on clinical examination, measurement of serum tumour markers (AFP, HCG), chest radiographs and CT scan of the chest and abdomen with and without bilateral pedal lymphangiography. In clinical stage I disease there is no evidence of tumour spread demonstrated by these investigations. When postorchiectomy tumour marker levels remain elevated involvement of retroperitoneal lymph nodes or metastatic spread elsewhere is found in all cases [8]. One way to evaluate clinical staging is to compare the results of investigations with the results of surgical staging (that is RPLND). Table 1 summarises findings from different studies [9–23]. Three conclusions can be drawn from this table: (i) the false negative and false positive rates of lymphangiography and abdominal CT scan are approximately equal to 30 and 15%, respectively. (ii) From the data in the

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