Mitotane Associated With Cisplatin, Etoposide, and Doxorubicin in Advanced Childhood Adrenocortical Carcinoma

Mitotane Monitoring and Tumor Regression

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Purpose: To define a mitotane dose for pediatric patients with adrenocortical cancer (ACC) that maintains therapeutic plasma levels (TL) between 14 and 20 μg/mL and to verify its antitumor efficacy in association with 8 cycles of cisplatin, etoposide, and doxorubicin (CED).

Methods: Powdered mitotane was dissolved in a medium chain triglyceride oil and administered to 11 children with ACC (2.4 to 15.4 y of age); an initial low dose was increased to 4 g/m^2/d. Ten of the 11 children had a germline *TP53* R337H mutation. Mitotane plasma levels were determined using high-performance liquid chromatography.

Results: The mitotane dose to maintain TL in 7 patients ranged from 1.0 to 5.3 g/m^2/d. Six children reached mitotane levels of 10 μg/mL in 3.6 months (1.5 to 5.0 mo), whereas 5 children took 8 months (6.5 to 12.5 mo). Minor to partial tumor remission was found in 5 patients (< 1 y) and complete remission was found in 2 patients. Of the 3 patients who are alive at the time of report, 1 patient has been without disease for 16 months, and 2 patients have progressive disease. All patients had recurrent metastatic disease (2 to 9 times). Mitotane toxic effects were nausea, diarrhea, vomiting, neurologic alterations, gynecomastia, a rare case of hypertensive encephalopathy, and CED-related hematologic toxic effects.

Conclusions: Mitotane daily dose to maintain TL is variable and monitoring should start 1.5 months after the beginning of treatment. CED combined with mitotane is the best available pharmacologic treatment for ACC, but further studies are required to characterize different profiles of therapeutic response.

Key Words: mitotane, adrenocortical cancer, children, chemotherapy toxicity, *TP53* R337H mutation

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Adrenocortical tumors are rare around the world, but the incidence in Southern Brazil is remarkable; 10 to 15 times higher[1] and in a more recent study, 3.5 per million children younger than 15 y of age due to the *TP53* R337H mutation.[2]

The clinical and pathologic characteristics of childhood adrenocortical carcinoma (ACC) documented in Southern Brazil are similar to those in other continents.[3,4] Complete surgical resection of local or regional disease, must be attempted and is the only curative approach. Ipsilateral retroperitoneal lymph node dissection is suggested for all stage II patients and is required in all stage III and IV patients.[4,5] However, this approach is not fully supported for all patients in another multi-institutional trial that includes mainly adult patients being evaluated prospectively (http://www.firm-act.org/committee.asp).

The use of mitotane is controversial as adjuvant therapy in stage I and II ACC, due to the lack of

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convincing data that the drug can prevent tumor recurrence, but it has been indicated in stage III and IV ACC in association with cisplatin, etoposide, and doxorubicin (CED),[5] as previously recommended for adult patients.[6] Many large studies of ACC patients consist mostly of adults and further studies are necessary to optimize mitotane use in children. There is not a consensus about dosage and duration of therapy in children. Two studies used mitotane 5 to 12 g/m^2 in 24 children, and had a therapeutical response rate of around 30%[7,8]; similar to studies in adults.

Owing to its lipophilic nature, mitotane is primarily stored in fat-containing tissues and, to a certain extent, crosses the blood-brain barrier and accumulates in the central nervous system.[9] Only 60% of mitotane is absorbed after oral administration, and damage to the mucosa may cause nausea and vomiting. Dosing around 6 to 10 g/d caused gastrointestinal side effects in 80% of patients and neuromuscular dysfunction or psychiatric symptoms in 40%.[9,10] Neurologic side effects are common (and severe) at serum levels higher than 20 μg/mL, and are usually reversible with dose reduction or discontinuation of treatment.[9] Two studies[11,12] have mixed powdered mitotane with cellulose acetilftalate, and claim to have reduced gastric intolerance; however, it reduced bioavailability as well. Other researchers[10] have tested commercially available tablets, enteric-coated gastric-resistant granules in capsules, and powdered tablets mixed with melted chocolate, emulsion (peanut oil, arabic gum, and water), or vegetable oil plus fat-free milk powder. The mitotane plasma levels were compared after standard doses were given in 2 different schedules: a single dose of 2 g, and in a long-term study, after a total dose of 200 g. These authors have concluded that the administration of mitotane with lipophilic vehicles increased mitotane absorption (with higher plasma levels) and reduced side effects.[10]

Mitotane belongs to a class of drugs that requires metabolic transformation for therapeutic action; although this may vary among species.[13–15] A retrospective study involving 34 adult patients suggested that the antitumor action of mitotane is observed at plasma levels around 14 μg/mL,[16] which was confirmed by another group in adult patients.[17] These 2 groups have reported extended survival and tumor regression in about 55% of patients with plasma levels higher than 14 μg/mL, showing a good correlation between mitotane plasma levels and survival. On the basis of these results, more recent studies have adopted the same mitotane plasma level (14 μg/mL) as the lower limit necessary for possible therapeutical efficacy.[6,9,12,18] Seven patients (aged 45 to 62 y), receiving mitotane at 1 to 3 g/d, took 3 to 5 months to reach levels above 14 μg/mL; an accumulated dose of 283 to 387 g.[19] However, in another study, 24 patients (20 to 76 y of age) taking 6 to12 g/d reached the same plasma levels after 2 to 11 months of treatment; corresponding to an accumulated dose of 207 to 2196 g.[12] It was noted that, after prolonged mitotane administration, the daily dose required to maintain levels of 10 to 20 μg/mL is substantially reduced, with some patients requiring as little as 500 mg to 1 g.[20]

Mitotane causes cytotoxic atrophy of the adrenal cells (both normal and neoplastic) by acting at the mitochondria, leads to adrenal insufficiency in most patients, and increases metabolic clearance of corticoids.[21–23] To avoid adrenal insufficiency, oral glucocorticoid replacement (preferentially a synthetic steroid more potent and with longer duration of action than hydrocortisone; eg, prednisone) and a mineralocorticoid (fludrocortisone) must be given, and the patient should be closely watched whenever supplements are necessary; especially during stress.[20,24–26]

The prospective study reported herein was designed (1) to monitor mitotane plasma levels using high-performance liquid chromatography, to define the required daily dose/m^2 to maintain a plasma concentration between 14 and 20 μg/mL in pediatric patients; (2) to verify whether there is a relationship between the time required to reach therapeutic plasma levels and body mass index (BMI); (3) to identify the plasma level at which mitotane may inhibit adrenal steroid synthesis; and (4) to define tumor regression caused by mitotane in association with CED.

MATERIALS AND METHODS

Patient Treatment and Monitoring

This prospective study was approved by the Ethics Committee from Erasto Gaertner Hospital and the National Cancer Institute/RJ. Fourteen children with ACC stage III or IV were initially enrolled from May 2003 to November 2004, after obtaining written informed consent signed by one of the parents. However, 3 patients were excluded from this study (2 died in the beginning of the study and 1 patient was referred by another institution without complete clinical data). The remaining 11 children were included in this study; 3 boys and 8 girls, ranging from 2.0 to 11.2 years of age at diagnosis (Table 1). Stage at diagnosis varied: I (n = 1), II (n = 2), III (n = 3), and IV (n = 5), presenting only virilization (n = 8), or virilization with Cushing syndrome (n = 3) (Table 1). There were 2 ACC cases that were recurrences from stage II (patients no. 6 and 9), 3 cases at stages III, and 6 cases at stage IV. One of the stage IV patients (patient no. 7) had primary tumor in the second adrenal gland, 12 years after a first tumor was diagnosed in the contralateral gland in stage I. Owing to the unusual interval of time between the 2 tumors (and their location), this second stage IV tumor was considered as a new tumor. Cava invasion was initially identified in 6 cases.

Compliance to treatment was the eligibility criterion adopted for accurate mitotane monitoring. In 10 children, mitotane was administered concomitantly with other antineoplastic agents (8 cycles of CED[6]), continued between the cycle intervals, and after completing all cycles, according to disease progression. Other cycles were added to 3 patients who had not yet completed the expected cumulative doses: CED (n = 2), or only cisplatin

TABLE 1. Clinical Features and Pharmacokinetics of Mitotane Accumulation Compared With Daily Dose

Patient	Sex	Age at Diagnosis	Age at Mitotane Start	Clinical Presentation	Initial BMI (kg/m^2)	Time to Reach Mitotane Plasma Level 14 ± 2 µg/mL (mo)	Mitotane Dose (g/m^2 BSA) When Mitotane Plasma Level was 14 ± 2 µg/mL	Mitotane Dose (g/m^2 BSA) to Maintain Therapeutical Levels (14 ± 2 µg/mL)	Highest Mitotane Plasma Level (µg/mL)
1	M	9 y 4 mo	12 y 11 mo	V	20.6	2.5	1.6	2.1	23.0
2	F	7 y 10 mo	7 y 10 mo	V	16.8	4.0	3.5	4.0	29.6
3	M	3 y 11 mo	6 y 4 mo	V	15.4	1.5	3.5	1.0	25.9
4	F	6 y 2 mo	6 y 2 mo	V	20.7	4.5	5.2	2.3	25.9
5*	F	11 y 3 mo	11 y 3 mo	V+CS	25.2	6.0	1.8	1.8	12.7
6*	F	4 y 4 mo	4 y 4 mo	V	16.6	6.5	4.1	4.1	15.9
7	F	2 y	15 y 10 mo	V	18.9	5.0	2.9	4.3	13.8
8	F	5 y 6 mo	6 y 2 mo	V	12.7	7.0	6.8	5.3	25.9
9*	F	9 y 9 mo	11 y 5 mo	V+CS	14.1	15.0	3.8	1.9	25.8
10*	M	2 y	2 y 4 mo	V+CS	16.6	10.0	7.3	3.3	28.2
11*	F	3 y 3 mo	3 y 7 mo	V	17.7	8.5	3.6	3.9	18.0
Mean	—	—	—	—	17.8	6.4	—	—	—
Standard deviation	—	—	—	—	3.5	3.8	—	—	—

*Patients taking mitotane for 3 to 10 mo before this study began.
CS indicates Cushing syndrome; F, female; M, male; m, months; V, virilization; y, years.

and doxorubicin (n = 1). One patient (patient no. 3, Table 1) received mitotane treatment twice: first for 8 months together with chemotherapy (as described above); and 14 months later (after recurrence was noted) for 6 months (mitotane was monitored only in the second period of treatment).

The chemotherapy regimen was adapted from Berruti et al,[6] as follows: cisplatin 40 mg/m^2, IV, 4 hours, days 1 and 9; etoposide 100 mg/m^2, EV, 1 hour, days 5 through 7; doxorubicin 20 mg/m^2, EV, 1 hour, days 1 and 8; mitotane 4 g/m^2/d. Mitotane was given orally at the starting dose of 0.5 to 1.0 g/d, and increased in increments to reach the target dose of 4 g/m^2/d within 2 to 4 wk.

Mitotane tablets, containing 500 mg each (Lysodren, Bristol-Myers Squibb Co, 345 Park Avenue, NY 10154), were powdered and dissolved in a tasteless MCT oil (Medium Chain Triglycerides, Royal Numico Group, SHS International, SHS North America, 9900 Belward Campus Dr Suite 100, Rockville, MD 20850). Each gram of mitotane was dissolved in 2 mL of MCT oil and this solution was added to other fat containing food, such as milk, chocolate milk, or yogurt. Two patients with sustained nausea and vomiting had to receive mitotane through a nasogastric tube, during 2 wk and 18 months, respectively. Mitotane was administered orally at a starting dose of 0.5 or 1.0 g daily, divided into 3 intakes. The targeted daily dose for the first months was 4 g/m^2/d (3 times a day), which was reached at weekly increments. Mitotane plasma levels were measured initially every 2 to 4 wk until reaching therapeutic concentrations (14 µg/mL), after which adjustments of dose and more frequent monitoring of mitotane plasma levels (every 1 to 2 wk) were performed. Patients with severe toxicity were managed by reducing the dose or transiently interrupting mitotane treatment.

A physical examination and hematologic profile were conducted before each course of chemotherapy. White blood cell, differential, and platelet counts were recommended between the twelfth and fifteenth day from the start of chemotherapy. Each patient underwent a regular follow-up, including routine biochemistry, endocrine work-up, and a physical examination. All patients were on daily corticoid replacement therapy (prednisone and fludrocortisone). Hormone screening included measurement of plasma adrenocorticotropic hormone (ACTH), androstenedione, 17-hydroxyprogesterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), testosterone, and cortisol, at intervals of 30 days. All patients received 24-hour intravenous hydration on the day of cisplatin treatment. In addition, ondansetron associated with dexamethasone or alizaprida were used routinely to ameliorate nausea and vomiting. Some patients who had previous episodes of vomiting were treated with intravenous hydrocortisone sodium succinate during chemotherapy, and intramuscular methylprednisolone sodium succinate after discharge, until normal food and fluid intake was documented.

Mitotane Assay

Mitotane assays were performed at LABAC (Curitiba, Brazil, www.cermen.com.br) using a modified protocol proposed in other studies.[27,28] Proteins from 250 μL of heparinized plasma were precipitated with 325 μL of acetone. After agitation for 60 seconds in a vortex mixer and centrifugation for 5 minutes at 5000 g, the supernatant was transferred to glass vials and the samples were injected into a high-performance liquid chromatography system from Varian, Inc (3120 Hansen Way, Palo Alto, CA 94304) with a quaternary pump (model 9012Q), diode array detector (model 9065), and automatic sampler (model AI200). Chromatography was performed on a Nucleosil C-18 column (4.6 × 250 mm) and the mobile phase was 85% methanol in 15% water, which was delivered at 1 mL/min. The ultraviolet detector was set at 239 nm. Each analysis was run in 7 minutes. Every sample was run in triplicate, and the results (μg/mL) were expressed as a mean.

Mitotane standard was purchased from The US Pharmacopeia Convention, Inc (US Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852). Stock standard solutions were obtained by dissolving 5 mg mitotane standard in 5 mL acetone (Merck & Co, Inc, One Merck Drive, PO Box 100, Whitehouse Station, NJ 08889). The calibration curve was prepared using 250 μL drug-free human plasma samples (blank plasma) spiked with known mitotane standard amounts to obtain 4, 10, 20, 30, and 50 μg/mL mitotane plasma levels; because the analytic range was estimated to be between 4 and 50 μg/mL. In each working day a new standard curve was created, and 2 quality control samples were freshly prepared from drug-free human plasma samples spiked with known mitotane concentrations. Every sample (from the calibration curve, spiked quality controls, and from patients) was analyzed in triplicate and the means reported. The efficacy of recovery obtained was 95%. Blood samples were obtained in heparinized tubes every 1 to 4 wk, according to side effects, changes in dosage, and mitotane plasma concentration. Plasma was immediately stored at −20°C until assayed.

Steroid Determination

Blood samples were obtained in heparinized tubes every 30 days and plasma was immediately stored at −20°C until assayed. Testosterone and DHEA-S were measured by chemiluminescent enzyme immunoassay (Diagnostic Products Corporation, 5210 Pacific Concourse Drive, Los Angeles, CA 90045) and cortisol (Diagnostic Products Corporation, 5210 Pacific Concourse Drive, Los Angeles, CA 90045) was measured by competitive immunoassay.

Genetic Profile of the ACC Children

Ten of the 11 children in this study had the germline *TP53* R337H mutation (1 child tested negative for this mutation), and loss of heterozygosity was found in each of the 7 tumor samples tested; as described in other reports.[29,30]

Statistics

It was verified whether the highest mitotane plasma levels were correlated with the total cumulative dose. A linear regression was performed to compare the mitotane dose and its relation with BMI. Quantitative variables are presented as means and were compared using the Student *t* test.

RESULTS

Treatment and Mitotane Monitoring

At the time of submission of this paper, 3 of the 11 patients remain alive. One patient has been free of disease for almost 2 years and seems to be in complete remission. The remaining 2 patients who are alive have progressive disease. Of the 11 patients treated, complete resection of the primary tumor was possible in 5 children, and complete resection of first metastasis was possible in 5 children. Despite these efforts, recurrence was observed in all patients. This was followed by new resection in 7 patients. Partial tumor remission was found in 5 patients for a short period of time (< 1 y).

Eight patients reached the initial target dose (4 g/m^2) of mitotane within the first 8 wk of treatment. The patient's individual doses were changed according to the presentation of toxic effects or when therapeutical levels were reached. The remaining 3 patients did not reach the initial target dose because of either chronic renal insufficiency (n = 1), toxic effect (n = 1), or because they rapidly reached the therapeutical level within 45 days (n = 1). When therapeutic levels were reached, mitotane doses ranged from 1.6 to 7.3 g/m^2 (n = 11), which were further changed to 1.0 to 5.3 g/m^2 (n = 11) to maintain therapeutical levels (14 ± 2 μg/mL) (Table 1). Four of the 11 patients studied were regularly taking mitotane (4 g/m^2, 5 to 12 mo) before beginning this study; a period when mitotane was not being monitored. The first evaluation of the mitotane plasma levels of these patients (day 1 of this study) revealed mitotane plasma levels ranging between 2.2 and 6.7 μg/mL and they continued in our study for 4 to 17 months.

During the study, the patient's mitotane plasma levels were monitored for a period ranging from 5 to 25 months, including retrospective analyses from 4 patients who were already on mitotane, 4 g/m^2/d, 1 to 8 months before initiating this study. For some patients (n = 4) it was necessary to discontinue treatment for 7 to 14 days once or twice before reaching the target plasma levels (14 to 20 μg/mL) due to gastrointestinal and neurologic toxic effects of mitotane (abdominal pain, nausea, vomiting and/or diarrhea). Patients no. 9 and 10 did not use the MCT oil during the first 13 or 7 months of treatment, respectively; whereas patients no. 7 and 8 had difficulties ensuring compliance to the exact mitotane daily dose and the MCT oil used during part of the follow-up.

The observed time to reach the therapeutic levels had a wide variation in 11 patients; ranging from 1.5 to 15 months, with a mean of 6.4 ± 3.8 mo, median of

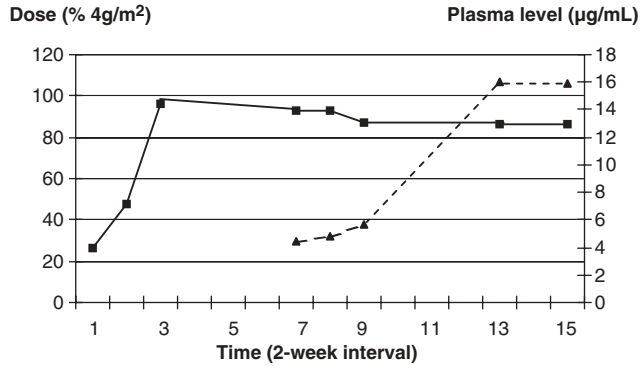

FIGURE 1. Time to reach therapeutical plasma levels (–▲–) are shown (data from patient no. 6; patients no. 6 and 8 were similar), which represent the intermediary time between the shorter period of time (for patients no. 1 to 4, and 7), and the higher (patients no. 9, 10, and 11). Mitotane plasma levels are compared with daily dose (–■–) represented as a percentage of the initial dose every patient was receiving (4 g/m^2).

6 months, and mean cumulative mitotane dose expressed by body surface area (BSA) of 466 ± 467 g/m^2 (ranging from 88 to 1509 g/m^2). In Table 1, each corresponding maximal plasma level is compared and shown with the respective clinical characteristics of the 11 children.

The initial proposed dose was changed in the second month of treatment, according to side effects (initially nausea and sporadically vomiting) or when plasma levels were above 20 µg/mL. Eighty-two percent of patients (9 of 11 children) reached mitotane plasma levels > 14 µg/mL. When the targeted therapeutic mitotane levels were reached (14 to 20 µg/mL), the daily dose required to maintain these levels was adjusted to 1.0 to 5.3 g/m^2/d. Four patients did not have a significant change in daily dose (4 g/m^2/d), and 1 continued on 5 g/m^2/d without reaching levels > 14 µg/mL (a teenager not regularly compliant to treatment, due to diarrhea). In 8 patients (patients no. 1 to 8), after a period of 0.8 to 6 months of very slow incremental increases in plasma concentrations, a fast rise (around 25 to 42 d) in mitotane plasma levels was observed from 10.7 ± 3.4 µg/mL (mean ± standard deviation) to values above 14 µg/mL (Fig. 1). The remaining 3 patients (patients no. 9 to 11) initially had a very slow increase in mitotane levels (lasting 7.5 to 14 mo; due in part to nonregular use of the exact daily dosage of mitotane and MCT oil), followed by a fast increase phase of only 30 to 52 days to reach values above 14 µg/mL. Similarly, the cumulative mitotane dose to reach a plasma concentration of 14 µg/mL was different for both groups: 88 to 1165 g/m^2 (patients no. 1 to 8) and 819 to 1410 g/m^2 (patients no. 9 to 11).

Considering that mitotane accumulates in fatty tissue, an association with BMI was investigated. These data are presented in Figure 2; a comparison between the BMI of each patient and the time required to reach approximately 14 µg/mL of mitotane in the plasma. Mitotane was able to inhibit steroid synthesis at plasma

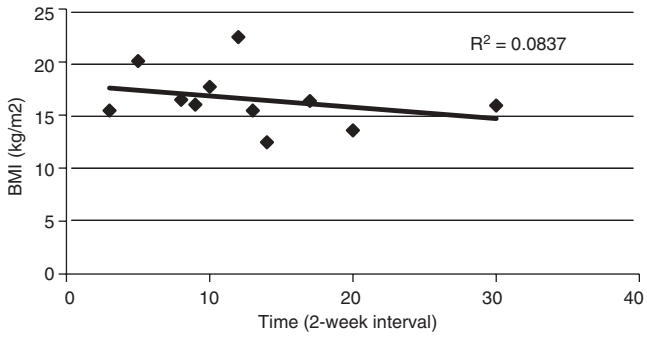

FIGURE 2. Correlation between BMI and the time necessary for mitotane to reach 14 ± 2 µg/mL in 11 patients.

levels starting between 5 and 10 µg/mL in 4 patients with undissected or partially removed tumor. A typical profile of this observation was shown in Figure 3A (testosterone) and Figure 3B (DHEA-S).

Although one of the objectives of this study was to describe the effect of mitotane on steroid synthesis, this information was only able to be captured in a subset of 4 patients studied. In the remaining 7 patients this was not possible (3 patients had no residual tumor when mitotane was initiated, and 4 patients were already on mitotane when this study was initiated). Mitotane was able to be shown to inhibit steroid synthesis in 4 patients with residual tumor; the hormones with progressive decrease were DHEA-S (n = 2), testosterone (n = 1), and cortisol (n = 1).

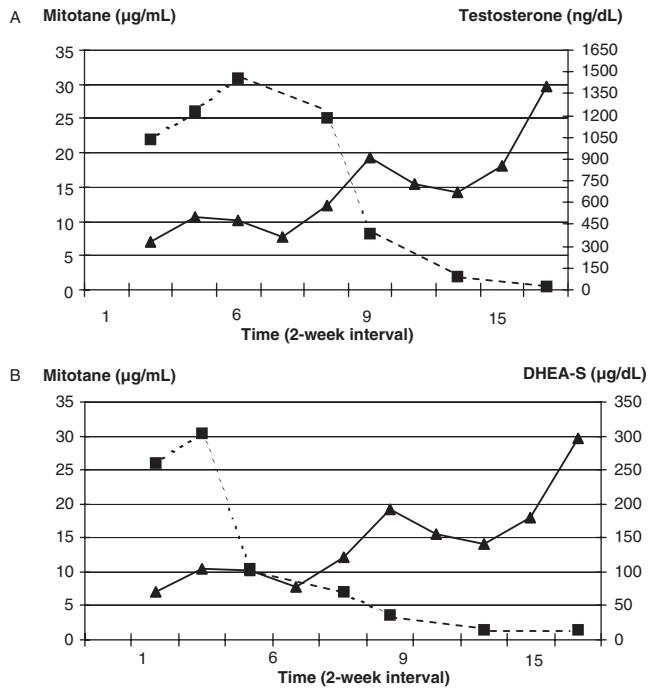

FIGURE 3. Typical pattern of steroid profile (patient no. 2) for testosterone (A) and in DHEA-S (B) levels. Comparisons are made between hormone (–■–) and mitotane plasma levels (–▲–).

Glucocorticoid replacement with prednisone ranged from 10 to 15 mg/m^2/d, which was constantly modified according to metabolic alteration caused by different daily doses of mitotane, stress induced by chemotherapy, infectious diseases, surgery, and clinical status. Monitoring of glucocorticoid dose was also guided by ACTH levels, which varied from 5.0 to 2570 pg/mL, 233 ± 488 pg/mL (mean ± SD). The ACTH levels above the median (57 pg/mL) usually were corrected by adjusting corticoid dose. Our experience with childhood ACT cell cultures treated with ACTH have not found increased cell proliferation and in some cell lines corticoid release did not occur in response to ACTH (personal communication; data not presented). Thyroid hormones and thyroid stimulating hormone were investigated several times during mitotane treatment and all results were normal, except for 1 patient who presented hypothyroidism before diagnosis of ACC, and who required a higher thyroid hormone replacement dose.

Toxic Effects

All patients had nausea at the onset of mitotane treatment. Four patients presented nausea and vomiting, but only 1 patient exhibited sporadic nausea with vomiting throughout treatment that did not change even at a very low daily dose of mitotane (1.0 g/m^2/d). Owing to gastrointestinal toxic effects, 3 patients had to receive mitotane through a nasogastric tube, and in both cases nausea and vomiting were controlled. However, a nasogastric tube was not tolerated by 2 other patients; 1 of them due to a congenital nasal septum defect associated with a prior upper respiratory infection. Other common toxic effects usually attributed to mitotane (neurologic alterations and gynecomastia) were observed in 3 patients. The patients with neurologic side effects (speech, lethargy, ataxia, and vertigo) associated with mitotane had plasma levels varying from 16 to 29.6 μg/mL—dose adjustments, combined with transient treatment interruption for 1 to 3 wk eliminated toxicity 1 to 2 wk later.

One of the patients died right at admission with gastrointestinal infection and adrenal insufficiency. The child's parents reported that she was sick at home for 1 wk and was on prednisone 12 mg/m^2/d (twice a day) and fludrocortisone 0.15 mg/d, and did not consider the recommended corticoid increments under these conditions. Signs and symptoms of adrenal insufficiency were noticed in 5 patients in the study who were promptly treated with intravenous hydrocortisone and hydration before discharge with normal food and fluid intake and oral corticotherapy replacement.

Patient no. 8 presented a rare mitotane side effect (high blood pressure followed by encephalopathy) previously described as an infrequent adverse effect,[31] that was controlled when mitotane treatment was temporarily interrupted and resumed at a lower dosage. All the above mitotane toxic effects were noticed in periods with or without other medications.

It has been reported that mitotane may cause prolonged bleeding time due to a reduction in platelet aggregation.[32] In the present study only 1 patient may have presented bleeding prolongation, most probably due to warfarin treatment prescribed after cardiac surgery that involved opening of the cava.

Although cisplatin may cause severe forms of kidney impairment or hearing loss, slight trouble hearing, or other hearing symptoms (ringing in the ears) were observed only in those patients with cancer recurrence who had additional doses of cisplatin, whereas renal function was slightly altered (13% in grade 1). Toxic effects observed during chemotherapy were described according to toxicity grade (World Health Organization) on cells of blood, liver, and kidney, as well as in metabolism (Table 2). No toxic death was observed during the 8 cycles of CED chemotherapy with mitotane.

Outcome

Despite complete resection of local and distant metastases, including retroperitoneal lymph node dissection (which is current protocol for stages II, III, and IV[5]) attempted in 9 patients and associated with a very aggressive antitumor therapy leading to partial (n = 5) or total remission of metastases (n = 1), only 3 of the 11 children (27%) are alive at the time of submission of this report; 1 child has been free of disease for the last 2 years (a survival time of > 7.5 y), and 2 children remain with progressive disease (survival of 2.9 to 4.4 y) (Table 3). Ten patients (90%) had 2 to 12 relapses, either during or after chemotherapy and mitotane treatment.

The cause of death of 4 children was disease progression. Four other children died due to other problems; the causes of death included surgical complications a few hours after the tenth surgical procedure (complete resection of lung metastasis), severe infection associated with adrenal insufficiency, brain hemorrhage during anticoagulation therapy after a surgical procedure to remove a caval thrombus, and one due to an unknown cause at home. A few weeks before surgery, the patient with brain hemorrhage presented normal platelet counts, bleeding times, and global coagulation parameters; excluding mitotane toxic effects that cause prolonged bleeding time.[17] According to the mother, the child who died at home (patient no. 11) went to bed apparently well and was not examined by a doctor. Only 1 patient has been in remission for almost 2 years (patient no. 1, who has already experienced 2 lung relapses). Two other remaining patients had recent relapses (Table 3).

The antineoplastic effect of mitotane in association with CED was observed in 6 patients with different degrees of remission: minor (n = 4), partial (n = 1), and complete (n = 2). However, only 1 of the 2 patients with complete remission survived and is without disease for almost 2 years. The other 2 patients who have survived, have progressive disease.

Patient no. 1 had resection which was only possible after treatment with mitotane. Only 4 (patients no. 3, 8, 10, and 11) of the 9 patients with advanced primary

TABLE 2. Chemotherapy Toxic Effects at Nadir

Toxicity Grade (WHO)	Liver Function* (%)	Kidney Function† (%)	Sodium (%)	Potassium (%)	Glucose (%)	Calcium (%)	Magnesium (%)	Hemoglobin (%)	White Blood Cells (%)	Platelets (%)
0	83	87	83	71	67	34	75	13	29	56
1	17	13	11	25	22	40	8	26	21	13
2	—	—	6	2	—	21	13	40	31	13
3	—	—	—	—	11	5	4	18	12	11
4	—	—	—	2	—	—	—	3	7	7

*ALT and AST.
†Urea and creatinine.

tumors (stage III and IV) were completely resected in a first surgical approach. Interestingly, patient no. 7 presented a second ACC in stage IV in the remaining adrenal gland, unrelated to the first ACC in stage I observed and completely resected 12 years before.

DISCUSSION

The current practice of our protocol administers a priori the 4 g/m^2/d dose of mitotane to all children, followed by individualized dosing on the basis of the target mitotane blood levels (14 to 20 μg/mL). This seemed to be a suitable strategy for the optimization of mitotane use in children as shown by the final wide range of dosages necessary to maintain therapeutic levels 2 to 5 months later (1.0 to 5.3 g/m^2). Eighty-two percent of the children (9 of 11 patients) reached mitotane plasma levels above 14 μg/mL. This is higher than the results observed in other studies in adult patients: 58%,[12] 57.1%,[20] and 48%.[17]

Heterogeneity in the therapeutic daily dose of mitotane has been attributed to several factors (including body weight, age, and metabolic factors), but poor adherence to treatment, intolerance, and large interpatient differences in blood concentration after wide dosing regimens in children and adults (2 to 8 g/d) also represent important reasons for the lack of consensus regarding a standard daily dose.

The association of mitotane with CED used in the present study was based on a previous study in adult patients,[6] but the present prospective study is the first one to test this protocol in children. CED combined with mitotane and surgical resection of recurrent disease seems to be relatively effective in increasing survival time, but it was not able to prevent recurrent metastatic disease in all children of our study. Only 3 patients had a more favorable outcome; 1 patient alive at 91 months after diagnosis and less than 2 years without disease, and 2 children with disease surviving less than 5 years.

Although these are the most effective known chemotherapy agents currently in use for childhood ACC, future investigations may find a profile of patients who may optimally benefit from this therapy. In a prospective multicenter phase 2 study of 72 adults (18 to 73 y)[33] taking proportionally much less mitotane than used in our study—their study parameters having been stated as: planned dose, 4 g/d (21 of 72 patients, 29%); maximum tolerated dose, 3 g/d (35 of 71 patients, 48%), 2 g/d (10 of 72 patients, 14%), and 1 g/d (5 of 72 patients, 7%)—in combination with 6 cycles of CED, tumor regression was observed in 35 patients (48%), including complete remission in only 5 patients (5 of 72 patients, 7%). Although we can say that their results, with complete remission were similar to ours (2 of 11 patients; but only 1 of 11 patients considering that only 1 patient survived), it is hard to interpret the authors' results. It would have been important in their study to evaluate mitotane daily dose and plasma levels, as well as duration

TABLE 3. ACC Evolution and Outcome Over a Period of 0.5 to 7.5 y

Patient	Stage at Diagnosis	Resection (Rs) of Primary Tumor at Diagnosis	Resection of Metastasis at Diagnosis	Response to 8 Cycles of CED & M	Duration of Mitotane Treatment	Relapse During or After CED & M Treatment	Evolution	Outcome (Survival in mo)
1	IV	CRs	Lung (CRs)	No disease during chemotherapy	19 mo	New lung metastasis during M/CRs + 6 cycles of CE + M	No disease after chemotherapy	AWD (91)
2	IV + Cava T	PRs	Lung and liver (inoperable)	MRe	16 mo	New lung metastases/M	MRe followed by PD	DOD (15)
3	III + Cava T	PRs	—	MRe	2 periods of 8 and 6 mo (second after fifth relapse)	Local (2 ×), lung (2 ×), mediastinum, kidney, other (3 ×)/CRs of all lesions + M	9 relapses	DOD (50)
4	IV + Cava T	Unresectable	Local, cava, lung, liver (unresectable)	CRe	9 mo	Lung/CRs + M	Relapse in inguinal region	DOD (18)
5	IV + Cava T	PRs	Regional LN (PRs)	Only M (MRe)	6 mo	Lung/only M	PD	DOD (7)
6	II	CRs	—	No chemotherapy	—	Lung/CRs + 8 cycles of CED + M (10 mo)	No disease after chemotherapy	DWD (infection and adrenal insufficiency) (12)
7	I/IV*	CRs	—	MRe	18 mo	Cava + liver (inoperable), lung (PR)	PD	DOD (47 mo after second ACC)
8	III	CRs	—	No disease during chemotherapy	20 mo	Local + cava T + liver + lung/CRs of lung lesions + M	PD	AD (35)
9	II	CRs	—	No chemotherapy	25 mo	4 lung relapses/CRs + 8 cycles of CED + M	New relapse in lung	AD (53)
10	IV + Cava T	CRs	Liver (inoperable)	PD	16 mo	Liver and cava T up to right atrium/only M	PD	DWD† (26)
11	III	CRs	—	No disease during chemotherapy	11 mo	Progression of lung metastasis/CRs + M	Relapse in lung	DOD (14)

*A girl with a rare second ACC in the second adrenal gland (stage IV) 12 y after first presentation in stage I in the contralateral adrenal gland (2 y old).

†Cerebral hemorrhage due to anticlotting medication (after vascular surgery to remove thrombus from cava).

AD indicates alive with disease; AWD, alive without disease; Cava T, cava thrombus; CED & M: cisplatin (C), etoposide (E), doxorubicin (D), & mitotane (M); CRs/PRs, complete or partial surgical resection; DOD, dead of disease; DWD, dead without disease; F, female; LN, lymph nodes; M, male. Responses to chemotherapy and mitotane: CRe indicates complete remission; MRe, minor remission; PD, progressive disease; PRe, partial remission.

of treatment, with the corresponding extension of therapeutic responses.

The small number of cases with Cushing syndrome (3 of 11 patients) compared with elevated levels of androgens in all of our 11 patients did not allow us to estimate a longer survival for children with virilization and/or poor prognosis for glucocorticoid secreting ACCs as previously reported.[4,33] There is no problem in dissolving powdered mitotane in tasteless MCT oil, which is considered a mode of delivery that diminishes gastrointestinal intolerance in most of the children (as reported by their parents). This vehicle may have contributed to the ability to maintain dosage as low as 1 g/m^2/d; sufficient to maintain therapeutic levels in a few patients.

The well known lipophilic property of mitotane encouraged us to evaluate interference of BMI in mitotane bioavailability; however, our results were inconclusive. The time required to reach therapeutic levels did not change with differences in BMI, which confirmed conclusions from a previous study.[12] However, because we have found a trend toward a shorter time to reach higher plasma levels in children with higher BMI, it would be interesting to test bioelectrical impedance, which is a more accurate parameter for analysis of fat mass. It was reported that all 7 patients (aged 45 to 62 y) taking 2 to 3 g/d had required a cumulative dose of 283 to 387 g to reach the therapeutic range 3 to 5 months later.[19] Taking into consideration the BSA for these 7 patients, the final average cumulative dose would be on average less than 120 g/m^2 (considering the average BSA for adults). Similarly, in a study involving 24 adult patients,[12] the final cumulative dose had a wider range (207 to 2196 g, or approximately between 70 and 750 g/m^2). More recently, the same findings were reviewed, showing that in some adult patients, administration of 2 g/d is sufficient to bring mitotane blood levels into the target range, whereas in others 5 g/d failed to reach target levels.[34]

Although these studies in adult patients present data similar to ours, there is a group of patients in our study with a proportionally higher cumulative dose that could be due to lack of compliance, lack of use of the MCT oil, or due to other metabolic differences. The discrepancy in the cumulative dose to reach the therapeutic level in plasma among the patients seems to be unrelated to age.

Our data should alert oncologists to the need for dose adjustments when mitotane plasma concentrations are around 10 μg/mL, due to fast further rise of mitotane levels (within 1 to 2 mo) and higher risk of undesirable side effects when plasma levels are around 20 μg/mL. During this period it is important to evaluate plasma levels twice a month.

Mitotane is able to inhibit steroid synthesis, a desirable effect that reduces endocrine manifestations in 75% of patients,[11,35,36] however, this may be one disadvantage in cases when the antitumor effect fails and ACC may continue growing and only is detected using imaging analysis. In this study, suppression of steroid production was observed at mitotane plasma levels starting between 5 and 10 μg/mL, as shown in Figures 3 A, B. ACC recurrence may cause only slight hormone level increases; sometimes below the upper limit for normal steroid levels. This was observed in patient no. 3 in 2 recurrences (data not shown), implying that steroid synthesis by the contralateral normal adrenal cortex was blunted and that further increases must have originated from metastasis.

Despite all surgical and pharmacological attempts to eradicate advanced stage of ACC (III or IV), its prognosis is still very poor. Future pharmacogenomics and in vitro tests may be able to predict mitotane efficacy and toxic effects, and should prevent undesirable use of this toxic drug for a large proportion of ACC patients. Most studies (Table 4) have reported on average a modest 15% to 25% regression of ACC in response to mitotane, which is similar to our present observation for the combination of mitotane, CED (Table 3). In addition, some tumors that responded to mitotane still underwent resistance and recurrence. As in the present study, initial control of hormone excess was possible in the majority of patients with incomplete tumor resection, and this may be a life-saving alternative for a subset of patients experiencing high blood pressure caused by direct and indirect glucocorticoid and mineralocorticoid effects, especially when they are not responding to other antihypertensive drugs.

A number of studies claim variable therapeutic responses (Table 4); however, some of them also include patients with localized disease. As shown in Table 4, there is no consensus for the type and dosage of corticoid replacement in most of the studies. In addition, replacement therapy is quite variable in quality (type and half-life of corticoid) and quantity; this may cause a problem for those who need to make a choice in their protocols.

Adrenal insufficiency was the cause of 1 death in the present study (Table 3), and it was observed in different degrees 1 to 3 times in 5 of the 11 children studied. As previously described, 2 of 21 children previously on mitotane died due to adrenal insufficiency,[8] implying that tight monitoring of corticoid replacement should be the primary focus for successful long-term management of children on mitotane treatment. Children may have limited oral absorption of corticoids taken during crises of vomiting due to the toxic effect of mitotane, which may lead to a lower systemic corticoid bioavailability, and this situation may lead to adrenal insufficiency, especially in those patients under strong antisteroidogenic influence caused by high circulating levels of mitotane.

In contrast, despite a low daily dose of hydrocortisone and its similarly potent analogs (with same duration of action), adrenal insufficiency has not been regularly reported in most of the studies in adult patients (Table 4), and this may be in part due to a difference in tolerability between children and adults to diminished function of corticoids. The current practice is to recommend prednisone (10 to 15 mg/m^2/d, in 2 or 3 intakes per day), or eventually an equivalent dose of a corticoid with longer half-life such as dexamethasone,

TABLE 4. Corticoid Replacement and Therapeutic Response to Mitotane and Other Chemotherapies

Author	n (Age in Y) and Stage	Protocol	Corticoid Replacement	Tumor Response
Berruti et al[33]	72 (18-73) Stages III and IV	M + E,D,C	Replacement not specified	Any PRe or CRe (35 of 72, 48.6%), only CRe (5/72, 6.9%)
Abraham et al[20]	36 (23-10) Stage IV	M + D,E,V	Hydrocortisone (20-120 mg daily) + fluorohydrocortisone (0.025-0.6 mg daily)	PRe or CRe (5 of 36, 22%)
Baudin et al[12]	24 (20-76)	M (group I)		Group I: CRe (1 of 13, 7.5%); PRe (3 of 13, 23%)
	Stages I-IV	M + surgery (group II)		Group II: CRe (3 of 11, 27%) Only patients with M plasma levels > 14 μg/mL achieved objective tumor response.
Kasperlik-Zaluska[37]	61 (13-70) Stages III and IV	M only (n = 55) M + 5-FU or M + C,E (n = 6)		M immediately after surgery: 18 of 32 (56%) survivors M with delay after surgery: 6 of 27 (22%) survivors
Berruti et al[6]	28 (27-66) Stages III and IV	M + E,D,C		CRe: 2 of 28 (7.1%); PRe: 13 of 28 (46.4%)
Haak et al[17]	62 (1-78) Stages I-IV	M or M + C,D,C or M + S		PRe or CRe (15 of 27, 55%), in patients with M levels > 14 μg/mL
Luton et al[11]	59 (6-81) Stage IV	M		PRe or CRe (8 of 37, 22%)
Decker et al[38]	36 (18-72) Stages III and IV and relapsed	M	Cortisone acetate (37.5 mg daily) + fludrocortisone (0.1 mg daily). One patient had AI and was not on mineralocorticoid replacement	PRe or CRe (8 of 36, 22%)
Khan et al[39]	40 (20-69) Stages I-IV	M + S	Hydrocortisone acetate (25-100 mg daily). 4 patients had AI	PRe or CRe (8 of 22, 36.4%)
van Slooten et al[16]	34 (20-71) Stage IV	M	Dexamethasone (1 mg daily) + fluorocortisone (0.1 mg daily) 4 patients had AI	PRe or CRe (8 of 34, 29%) In 7 of 8 patients with PRe or CRe, M levels were > 14 μg/mL
Teinturier et al[8]	21 (0.5-16) Stages I-IV	M, C,D, 5-FU, radiotherapy	Replacement not specified. 2 patients died due to AI	PRe or CRe (7 of 21, 30%)

AI indicates adrenocortical insufficiency; C, cisplatin; D, doxorucibin; E, etoposide; 5-FU, 5-fluorouracil; M, mitotane; PRe or CRe, partial or complete remission; S, streptozocin; SD, stable disease.

associated with fludrocortisone (0.15 to 0.2 mg/d) while on a high mitotane daily dose.

It is important to recommend the augmentation of glucocorticoid dosage (2 or 3-fold) during periods of stress. There was no case of a lethal toxic dose due to CED and/or mitotane, and toxic effects have already been previously reported. Toxicity was particularly more intense during CED in patients who were regularly taking mitotane, and in a period complicated by stress, which usually required additional attention and constant evaluation of the daily dose of corticoids.

One important decision taken in this prospective study surrounding plasma level monitoring was the need for adjustment of mitotane dosage in most of the patients when plasma levels crossed the barrier of 10 μg/mL; a critical level because a further increase in plasma levels of mitotane occurs rapidly. To minimize toxic effects (mainly neurologic ones) and to ensure better adherence to treatment, it is recommended to maintain mitotane plasma levels above 14 μg/mL as previously reported.[16,17] In our experience, control of mitotane excess could be achieved with plasma determination and dosage adjustment every month until reaching 10 μg/mL, and every 2 wk thereafter.

In line with other protocols suggested for adult patients,[34] treatment for children may be initiated with 0.5 to 1.0 g/d and the dose rapidly increased depending on gastrointestinal tolerance to 4 g/m^2/d; with measurement of mitotane plasma levels after 30 to 45 d, adjusting dose according to plasma target concentration and tolerability.

There is no consensus about the duration of mitotane treatment yet; however, we have to take into account that for most patients the first 2 to 5 months of mitotane treatment may not be clinically therapeutic in an antitumor role due to the low plasma levels.

We propose that a prospective study taking into account the therapeutic response according to the geographical and genetic background of patients would be very helpful in providing a further understanding of the optimal adjustment of dose and plasma monitoring for mitotane. We believe that ACC remission in response to the protocol used in the present study also depended on the overall genetic background of patients, age, and ACC genoma, and a larger number of children with ACC from different parts of the world should be investigated.

The significance of a germline *TP53* R337H mutation in almost all patients (10 of 11 children) and loss of heterozygosity in all of the 7 children tested, needs to be elucidated in terms of tumor malignancy as shown for other types of mutations[40] and response to non-surgical treatment, and should be compared with the results of treatment of children from other institutions where other types of mutations are more common.

According to our knowledge, this was the first reported prospective study to monitor mitotane plasma levels in children. This study was necessary to determine accurate dose adjustments and to prevent toxic effects associated with mitotane therapy. Although mitotane was effective in causing antisteroidogenesis in all children with residual ACC, its efficacy as an antitumor drug in childhood ACC is still very limited and further studies are required in combination with CED. Future projects on pharmacogenomics may determine and define the profile of patients who will respond favorably to mitotane treatment, and may prevent its use in those who would not benefit from this treatment.

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