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ABSTRACT

We conducted a retrospective study on the long-term effect of mitotane treatment on testicular adrenal rest tumors (TARTs) in 5 adult patients with classic 21-hydroxylase deficiency,. After 60 months of mitotane treatment, a decrease of adrenal steroids was observed in 4 patients. Testicular ultrasonography showed complete disappearance of TART in 2 patients, stabilization in 2 patients and a halving of TART volume in the remaining patient. Sperm count improved notably in 2 patients who had normal baseline inhibin B levels and small inclusions, thus enabling cryopreservation of the subjects' semen. Four years of follow-up of these two patients after the withdrawal of mitotane showed no recurrence of TART and persistent normal testicular function. In conclusion, mitotane could be used as a last resort in CAH patients in the cases of azoospermia associated with TARTs but normal inhibin B levels, as it can improve long-term endocrine and exocrine testicular function.

INTRODUCTION

Twenty-one-hydroxylase deficiency (21OHD) is the most common form of congenital adrenal hyperplasia (CAH). Male patients with CAH may present impaired testicular function and infertility (1). Testicular adrenal rest tumors (TARTs) have been described in these patients (1-4) and have been demonstrated to constitute one of the most important causes of infertility. In recent studies, the overall prevalence of TARTs in classic CAH patients was 40% (1-6). TARTs appear to be associated with poor hormonal control and concomitant elevated adrenocorticotropic hormone (ACTH) (7,8). Molecular characterization of TARTs has shown that these tumors have multiple steroidogenic properties, including the expression of adrenal cortex and typical Leydig cell markers (9), leading to the hypothesis of pluripotent cells as the origin of TARTs (3). To date, there are no methods to prevent the development of TARTs, nor are there guidelines to treat patients with TARTs (3). Systematic ultrasound evaluation is recommended at puberty to detect lesions at an early stage. A semen analysis should also be done as soon as possible and the question of systematic sperm cryopreservation seems fully justified (3). Treatment options for male patients with TARTs are still limited and mainly based on good hormonal control with glucocorticoids (3,10,11). However, long-term high doses of glucocorticoids do not always restore testicular function and often have unacceptable side effects (3).

Mitotane, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chloro-phenyl) ethane, has been used for several decades to treat adrenal carcinoma and Cushing's syndrome based on its potent adrenotoxic effects (12-14) and capacity to block cortisol synthesis by inhibiting 11ß-hydroxylation and cholesterol chain cleavage. Recently, Bry-Gaillard *et al*. have shown that mitotane could restore fertility in CAH patients with TARTs (15). In this study, we suggest that long-term mitotane treatment is able to shrink adrenal rest tumors, improve sperm count and adrenal secretion in 5 adult patients with classical CAH due to 21OHD presenting testicular

adrenal rest tumors in whom intensified glucocorticoid therapy was inefficient or impossible due to major side effects.

Patients and Methods

Patients

Medical files from 5 patients were recorded retrospectively. The main clinical features are summarized in Table 1. All patients were diagnosed as newborns with a salt-wasting form of CAH due to 21-hydroxylase deficiency. They were treated with hydrocortisone and mineralocorticoids. Patients 1, 3 and 4 were homozygous for an IVS2 splice mutation, patient 2 was compound heterozygous for a large lesion and L307fs and patient 5 was compound heterozygous for R386W and a large lesion. Testicular adrenal rest tumors were diagnosed by testicular ultrasonography in all patients except patient 4, in whom TARTs were diagnosed by clinical examination. Semen analysis showed complete azoospermia in patients 1 and 3 and oligospermia in patients 2 and 5. Semen analysis was not possible in patient 4 (anejaculation). None of these patients had fathered children at baseline. The 5 patients were treated with glucocorticoids and mineralocorticoids (figure 1) and had already increased their doses of glucocorticoid treatment without any efficacy in reducing TARTs volume. Furthermore, they had side effects of weight gain or osteopenia in response to the glucocorticoid therapy.

Each patient underwent regular follow-up, including routine biochemistry, endocrine work-ups, and physical examinations, to monitor the safety and efficacy of the treatment. The endocrine work-ups included fasting blood samples to ascertain levels of measurement of plasma 17OH-progesterone, testosterone (T), androstenedione (A), ACTH, renin, testosterone, sex hormone binding globulin (SHBG), LH, FSH and inhibin B, in the morning, before medications. Semen analysis was performed according to the World Health Organization (WHO) guidelines. Each patient had testicular imaging at 6 months, then at 12 months (except patient 3 and 4), 24 months

(except patient 4), 36 months and 60 months (except patient 2). A testicular sonogram was performed on each patient, except for patient 2 who underwent testicular magnetic resonance imaging (MRI). Treatment compliance was assessed by monitoring plasma mitotane levels every 6 months (14,16).

In France, retrospective studies such as ours do not require institutional review board/institutional ethics committee approval to analyze the data and publish the results.

Methods

Mitotane therapy

Mitotane treatment (Lysodren, HRA; 500 mg tablets) was proposed to the 5 patients. It was administered orally at a starting dose of 0.5 to 1 mg daily and then increased to 2 to 3 g daily or the highest tolerated dose. The dose was adjusted by monitoring plasma mitotane levels to maintain drug concentrations under 14 µg/ml, thus avoiding toxicity. The highest doses were 0.5 g/d in patient 1, 4 g/d in patient 2, 1 g/d in patient 3, 2.5 g/d patient 4 and 3 g/d in patient 5. Patients 1, 2, 3 and 5 were treated for 5 years. Patient 3 interrupted treatment for approximately 18 months following the occurrence of acute adrenal insufficiency. Patient 4 had to stop treatment after only 1 year due to poor digestive tolerance. During treatment, the dose of steroid coverage in the 5 patients was increased to prevent adrenal insufficiency. Discontinuation of treatment was decided according to the response to treatment (improvement or even normalization of sperm analysis, decrease or disappearance of inclusions on ultrasound), or in case of poor tolerance to treatment.

Data analysis

The results are presented as the mean±SD if not stated otherwise.

Results

Adrenal steroids and ACTH

As shown in Figure 1, 17OHP, progesterone and A levels were very high at baseline in 4 of the 5 patients. Only patient 3 showed a good hormonal balance under treatment, with 17OHP levels at 2.0 ng/ml and A levels at 0.65 ng/ml. During treatment, the 17OHP and A levels of patients 2 remain elevated. After 12 months of treatment, there was a median decrease of 88% of serum 17OHP levels and of 57% of serum A levels in the 3 remaining patients (Figure 1). Thereafter, hormonal control remained stable during treatment with mitotane, except in patient 2, in whom 17OHP, A and progesterone increased concomitantly with poor compliance with treatment. This was confirmed by the mitotane levels, which remained low during the first two years of treatment (<2 mg/l), (Figure 1).

Testicular function and morphological evaluation of TART

The evolution of testicular hormonal function is represented in Table 2. At the initial evaluation, basal serum LH and FSH levels were low in patients 1 and 5, indicating gonadotrophin insufficiency. After 24 months of mitotane treatment, there was a recovery of gonadotropin secretion, and total testosterone levels increased, due to elevated SHBG, which is generally associated with mitotane treatment. Patient 2 had moderate testicular insufficiency after 24 months of treatment. Patients 3 and 4 showed elevated gonadotrophin levels, reflecting testicular failure.

The results of testicular investigations are presented in Table 1 and Figure 2. Each patient had testicular imaging at 6 months, showing no change of TARTs volume. In patients 1 and 5, there was a near disappearance of the intratesticular masses by ultrasonography and MRI at 24 months, concomitant with the normalization of sperm count. By ultrasonography, there was a relative stability of TART volume during mitotane treatment in patients 3 and 4 and a halving of TART volume in patient 2.

The sperm count results before and during mitotane treatment are presented in Table 3. Patients 1 and 5 showed normalized sperm count during treatment, which allowed for the storage of their semen by cryopreservation. Patient 2 had severe oligospermia and azoospermia 6 months later. This patient had the largest TART. No sperm analysis was done during the therapy with mitotane in patients 3 and 4, for personal reasons.

Safety

Weight loss was observed in patients 1, 2 and 5, at respectively -8.1%, -15.7% and -18.4%. The weight remained stable for patient 3, and patient 4 gained 9.5 kilos in 6 months (+15.8% under treatment). Patient 4 had to stop the treatment prematurely due to poor digestive tolerance (nausea and diarrhea). Patients 3 and 5 each experienced one episode of adrenal crisis during treatment, respectively after 20 and 5 months of treatment, requiring a short hospital stay for each, followed by discontinuation of treatment in patient 3 and a simple adjustment of glucocorticoid treatment in patient 5. None of the patients require higher doses of fludrocortisone during therapy. Moderate elevation of LDL-c was observed during treatment, but this symptom resolved with reinforced dietary and lifestyle measures. No patients were treated with statins. Finally, hepatic cytolysis and minimal anicteric cholestasis <1.5N was observed in all patients except patient 2, with complete normalization after discontinuation of the treatment.

Long-term follow-up

Long-term follow-up after discontinuation of the treatment was available for patients 1, 4 and 5. Two years after the discontinuation of mitotane, patient 1 fathered a healthy child. His last ultrasound 4 years after the discontinuation of treatment found stable testicular volume, with minimal inclusions (0.1 cc on the right, 0.2 cc on the left) and hormonal assays showed normal testicular function (FSH 3.4 IU/l, LH 5.2 IU/l, TT 7 ng/ml, SHBG 60 nmol/l, inhibin B 178

ng/ml).

One year after discontinuation of mitotane, patient 5 showed a decrease in sperm count, nevertheless allowing for semen cryopreservation. The last ultrasound done 6 years after stopping treatment found testicles of normal volume without TARTs, and normal testicular function (FSH 3.2 IU/l, LH 5.4 IU/l, TT 9.5 ng/ml, SHBG 86 nmol/l, inhibin B 201 ng/ml).

Five years after the one year treatment with mitotane, patient 4 still had moderate testicular insufficiency (FSH 13.3 IU/l, LH 9.2 IU/l, TT 6.4 ng/ml, SHBG 38 nmol/l, inhibin B 33 ng/ml) with bilateral testicular hypotrophy by ultrasound (8 cc on the right, 10 cc on the left), and increased inclusion volumes (4 cc on the right, 4.5 cc on the left). Semen analysis five years after stopping treatment showed cryptozoospermia (4 spermatozoa).

Discussion

Until now, there have been no clear guidelines for the treatment or prevention of TARTs (3). The development of TARTs is generally understood to be the result of sustained elevation of plasma ACTH concentrations, usually associated with poor control of CAH. However, TARTs have also been reported in men with adequate control of CAH and, conversely, some subjects with poorly controlled CAH never develop TARTs despite chronically elevated ACTH levels. The first choice of treatment is intensification of glucocorticoid treatment to suppress ACTH, based on a few cases in which this has led to reduction of the tumor size and improved testicular function (17-20). Hydrocortisone, dexamethasone, and prednisone were all used as treatment for TARTs (21). However, no prospective studies of TARTs and intensified glucocorticoid treatment have been published.

Recently, Bry Gauillard *et al.* reported the case of one CAH patient with TART who was successfully treated with mitotane to restore fertility (15). In our study, we report five patients

with TARTs unresponsive to intensified glucocorticoid treatment, which for half of them were successfully treated with mitotane, thus demonstrating the long-term effect of mitotane to improve testicular function and fertility in CAH patients. Two of the five patients presented with hypogonadotropic hypogonadism due to poor hormonal control of CAH. The difficulty in diagnosing hypogonadism in men with CAH is related to the fact that testosterone measured in serum is a mixture of testosterone of gonadal and adrenal origin. Undetectable or low gonadotrophins, associated with high levels of androstenedione and progesterone, should help lead to this diagnostic. Recovery of hypogonadotropic hypogonadism was achieved in these patients under mitotane therapy, thanks to control of the disease. Interestingly, these patients had normal inhibin B levels, suggesting intact Sertoli cells, in accordance with a previous study (10). These patients therefore probably had impaired endocrine and exocrine testicular function due to poor hormonal control and recent TART development. We demonstrate that, in the early stages of TART development, mitotane therapy can lower ACTH levels, reduce TART size and improve testicular function and fertility. Inhibin B may be a good indicator of the success of mitotane therapy.

On the contrary, in the later stages of TART development, this approach may be effective at reducing tumor size and improving adrenal function or controlling further growth and testicular damage, but is ineffective at improving testicular function and fertility, due to irreversible damage of the testis. This was the case in patients 2 to 4, who presented testicular failure at baseline or discovered during the mitotane therapy. These patients exhibit low levels of inhibin B at baseline.

Mitotane is a metabolite of the pesticide and endocrine disruptor DDT (dichlorodiphenyltrichloroethane) and is classified as a teratogenic compound worldwide (12,13). However, little is known about its effects on human development or its direct effect on oocytes or sperm. A recent study on 4 women having conceived while taking mitotane, or with

detectable mitotane plasma levels, showed that exposure of these four children to mitotane in utero seemed to have no clear teratogenic effect (22). Two studies on reproductive toxicity of DDT in adult rats have showed direct testicular impact of these products, with a dose-dependent reduction of testicular weight and the number as well as the percentage of motile spermatozoa in the epididymis (23,24). Interestingly, our study shows the absence of TART recurrence and persistence of spermatogenesis in the two responsive patients far from mitotane withdrawal. Therefore, conception is safe at a distance from treatment.

However, mitotane does cause irreversible chemical adrenalectomy, and is therefore only recommended in patients who show good compliance to the adrenal replacement therapy. Glucocorticoid doses should be increased during this treatment because it induces CYP3A4 in a major way, leading to more rapid inactivation of hydrocortisone, requiring a 50-100% increase in GC dose to avoid adrenal crisis, as experienced by 2 of our 5 patients (25). None of our patients experimented hypogonadism symptoms during mitotane treatment, as it has been reported in patients with adrenocortical cancer (25). Finally, liver function and cholesterol metabolism must be closely monitored in these patients (26).

In conclusion, patients with CAH should be informed about the risks of the development of TARTs, and consequently the high risk of infertility. They should be monitored regularly by ultrasound and should be offered systematic semen evaluations and cryopreservation. Mitotane should be used as a last resort in men with 21-hydroxylase deficiency and azoospermia in whom azoospermia is associated with large testicular adrenal rest tumors despite normal inhibin B levels, as it can improve endocrine and exocrine testicular function with residual effects lasting several years after withdrawal.

Declaration of conflicts of interest

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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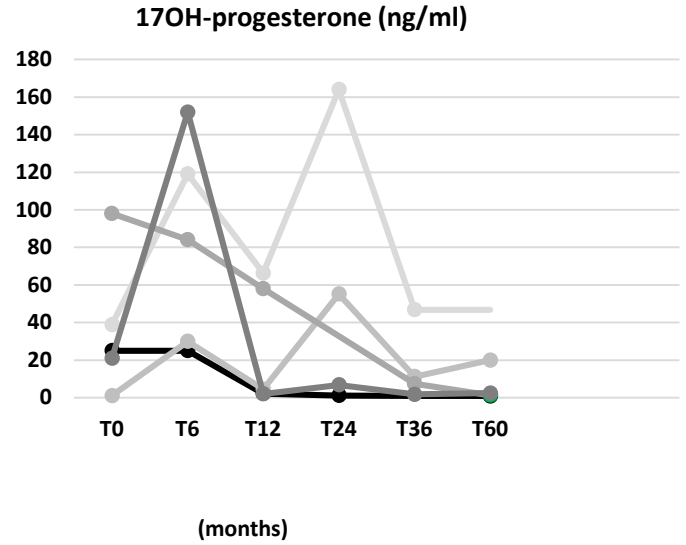
Figure Legends

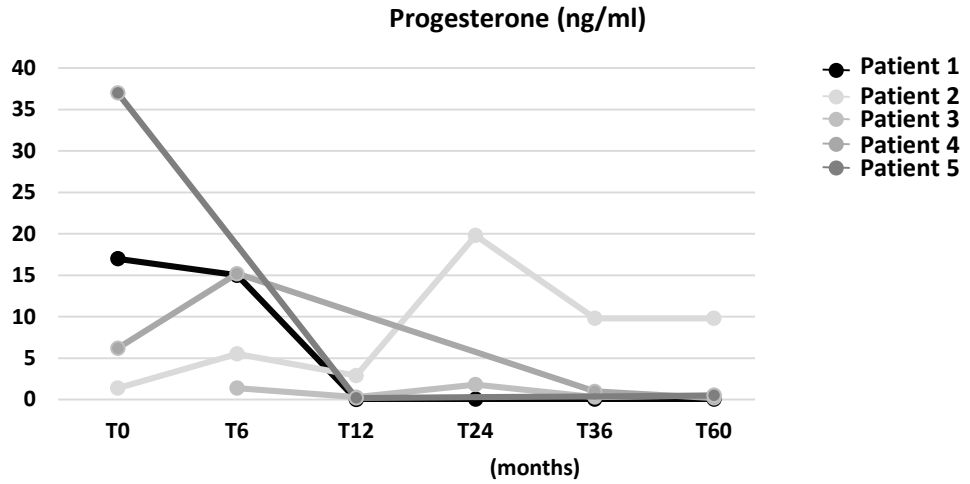
Figure 1 : Adrenal steroids, ACTH and mitotane levels at baseline and during mitotane treatment.

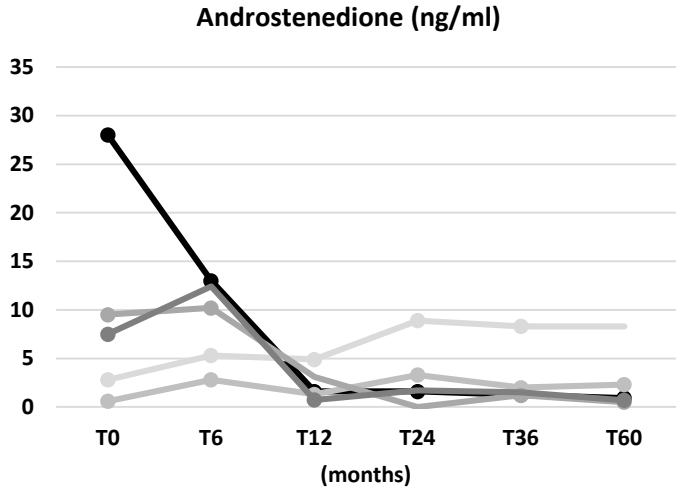
Normal baseline values : 17OH-progesterone (ng/ml) 0.6-3.4; Androstenedione (ng/ml) 0.2-2.9; Progesterone (ng/ml) 0.2-1.4.

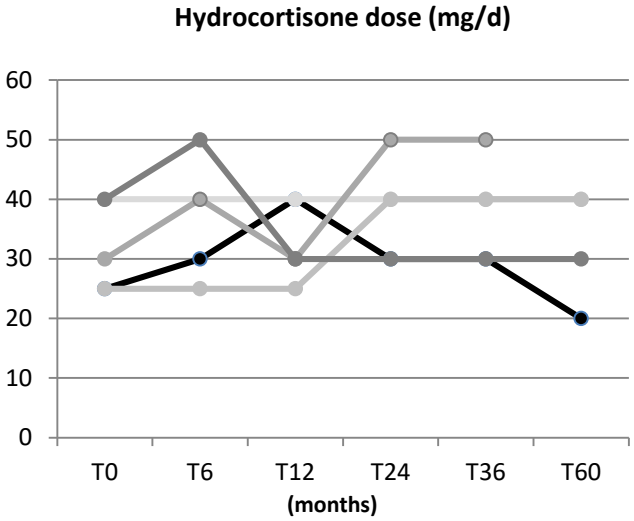
Figure 2 : 28 Year old man with adrenal hyperplasia who had bilateral testicular masses.

T2 weighted Axial MR (A, B) shows bilateral intratesticular masses that are hypointense compared with normal testicular tissues. Longitudinal sonogral of right testicule (C) shows large hypoechoic solid intratestcular masses with sound attenuation, avascular on Color doppler sonogram (D),









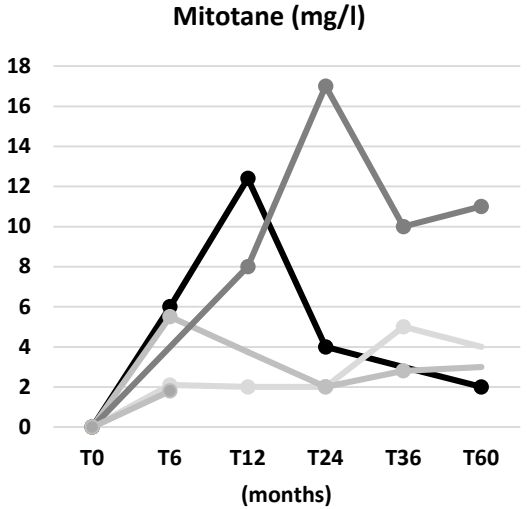


Figure 1 : Adrenal steroids, hydrocortisone dose and mitotane levels at baseline and during mitotane treatment
Normal baseline values :
17OH-progesterone (ng/ml) 0.6-3.4
Androstenedione (ng/ml) 0.2-2.9
Progesterone (ng/ml) 0.2-1.4

Table 2. Evolution of testicular function in CAH patients at baseline and during mitotane treatment

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
FSH (IU/l)					
Baseline	0.1	9.8	17	7.1	1.2
T12	3.3	ND	8.7	4.1	5.2
T24	7.5	8.1	13.1	ND	9.1
T36	6.7	14.4	18.1	15	ND
T60	3.6	ND	14.8	ND	8.2
LH (IU/l)					
Baseline	0.34	9.3	11.2	9.2	1.1
T12	9.1		11.4	4	4.3
T24	14	11.3	12	ND	15.2
T36	11.3	11.6	10.4	ND	9.8
T60	5.4	ND	10	9.8	2.7
Total testosterone (ng/ml)					
Baseline	5.7	8.8	5	6.2	3.4
T12	17.5	2.9	9.8	4.1	9.5
T24	19	7.3	9.3	ND	29
T36	9.3	11.2	11.0	3.7	17
T60	7.5	ND	12.8	ND	8.6
SHBG (nmol/l)					
Baseline	50	43	34	30	32
T12	220	68.4	78	ND	244
T24	128	113	46	ND	172
T36	77	142	61	27	124
T60	70	ND	81	27	87
Inhibin B (ng/ml)					
Baseline	128	131	15	31	167
T12	167	60	71	40	111
T24	ND	46	67	ND	ND
T36	ND	39	43	42	126
T60	152	75	72	33	128

ND: not determined
Normal baseline values:
FSH (IU/l) 1.5-12.4; LH (IU/l) 1.5-8.6; Total testosterone (ng/ml) 2.5-8.4; SHBG (nmol/l) 16.5-55.9; Inhibin B (pg/ml) 25-325

Table 1. Clinical and ultrasonographic characteristics of the 5 CAH patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (years)	24	20	21	28	24
Height (cm)	162	172	172	163	165
BMI (kg/m^2)	28	21	18	23	27
Treatment HC FC	 25 mg/d 100 µg/d	 40 mg/d 100 µg/d	 25 mg/d 50 µg/d	 30 mg/d 150 µg/d	 40 mg/d 100 µg/d
Testicular volume (left/right, ml)	7/5	18/16	9/11	19/25	12.5/13
TART volume (left/right, ml) Baseline 24 months 36 months 60 months	 0.8/1 0/0 0/0 0.1/0.2	 8/8 4/2.1 5/6	 5/6 4/6 5/4.5 4/6.5	 4/2.5 2.5/2.7 2.6/2.1	 0.3/0.2 0/0 0/0 0/0

HC: hydrocortisone; FC: fludrocortisone; BMI: body mass index; TART: testicular adrenal rest tumors.

Table 3. Evolution of sperm parameters in CAH patients at baseline, during mitotane treatment and after discontinuation of the treatment

Sperm count ($x10^6$/ml)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Baseline	azoospermia	1.2 $x10^6$/ml	0.06 $x10^6$/ml	anejaculation	15 $x10^6$/ml
At 12 months	12 $x10^6$/ml	azoospermia	ND	ND	azoospermia
Stop of mitotane	24 $x10^6$/ml	ND	ND	ND	39 $x10^6$/ml
After stop of mitotane (months)	33 $x10^6$/ml (16) 15 $x10^6$/ml (60)			4/ml (60)	7.2 $x10^6$/ml (12)