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Effects of Anti-inflammatory and Immunosuppressive Doses of Dexamethasone on Serum fT3 and fT4 in Ouled Djellal Algerian Ovine Breeds

Kellali Narimane[1*], Kerrour Mustapha[2], Lakhdara Nedjoua[1], Boudhene M Amir[1]

[2]Institut of Veterinary Science of Constantine, Route de Guelma el-khroub 05, 25000, Constantine, Algeria
[1]GSPA: laboratoire Gestion de la Santé et Productions Animales, Route de Guelma el-khroub 05, 25000, Constantine, Algeria

ABSTRACT

Synthetic Glucocorticosteroids (GCs) are widely used in veterinary medicine for their anti-inflammatory and immunosuppressive actions. However they are deemed to have multiple side effects such as hypothyroidism. The aim of this study was to determinate the effects of Dexamethasone (DEX) administered parenterally (IM) at therapeutic doses, conventionally used in the anti-inflammatory and immunosuppressive treatments on the thyroid function of sheep. Fifteen sheep of Ouled Djellal breeds were randomly divided into three groups of 5 rams. The animals received an injection of DEX (DEXALONEND solution) at a rate of 1.52 mg DEX/d for group 1 and 3.4 mg DEX/d for group 2 respectively, during 6 days; the third group has served as a control group and did not receive any treatment. Blood samples taken over a period of 3 weeks were analyzed to define the plasma levels of free thyroid hormones fT4 and fT3. It has been shown that Dexamethasone induced a significant decrease ($P < 0.05$) of serum fT4 and fT3 24 h following the beginning of the treatment. Hormone levels have returned to their baseline values 24 h after treatment withdrawal. From these results, we can deduce that DEX antithyroid effects are transitory and therefore do not have a significant impact on the health of the animals.

Keywords: Thyroid, Dexamethasone, Sheep, fT4, fT3

INTRODUCTION

Since their discovery, glucocorticoids have been widely used for their anti-inflammatory and immunosuppressive effects [1]. Like any drug, GCs have many side effects such as the possibility of interfering with thyroid function [2]. The disturbance of this gland can have serious effects on animal's health. In Algeria, sheep herds are subject for excessive use of synthetic glucocorticoids by clinical veterinarians. In addition, several authors have investigated the effects of glucocorticoids on human thyroid function [2-5] rats [6] and in dogs [7-12]. However, very few works have been done in sheep and therefore the effect of these substances on thyroid function in this species remains poorly explored. On the basis of these findings, it is essential to study the possible antithyroid effects of these drugs in local sheep.

MATERIALS AND METHODS

Experimental animals

Fifteen Ouled Djellal sheeps, aged 12-14 mon, weighing 45-53 kg were included in this study. All sheeps were clinically healthy and did not receive any medication prior to the experiment. The animals were randomly divided into three groups of 5 sheep each.

Experimental protocol

Two groups of sheep were injected intramuscularly with Dexamethasone (DEX) (DEXALONEND solution, 2 mg disodium phosphate of DEX, Coophavet, France) at a dose of 1.52 mg/day (Anti-inflammatory dose) for group I and 3.4 mg/day (Immunosuppressive dose) for group 2. The third group, which is the control group, received no treatment. Treatment with dexamethasone lasted 6 days (usual duration of anti-inflammatory treatment).

Samples collection

The blood samples were obtained from the jugular vein and collected in vacuum heparinized tubes. The samples were immediately centrifuged and the serums were stored at -20°C until analysis. The first sample was taken at 48 h before DEX administration. The second sample (1st one upon the treatment) was performed 6 h after the 1st injection of DEX. Between the 3rd and the 8th, the samples were collected at 24, 48, 72, 96 and 120 h after the beginning of the treatment. From the 9th to the 11th intake, the samples were taken at 1, 6 days and 14 days after the end of the

treatment. The same sampling protocol was adopted for the three groups.

Determination of fT4 and fT3

The analyzes were carried out within a period not exceeding four weeks. The assay was carried out by Chemiluminescent Microparticle Immuno Assay using the Architect ci8200 automaton (ABBOTT laboratory, U.S.A)

Statistical analysis

Data were analyzed using Turkey's test and ANOVA test (Minitab® 15). Differences were considered as significant when $P < 0.05$.

RESULTS

fT3 and fT4 concentrations in blood samples are summarized in (Tables 1 and 2). In control group (group III), the scattering of fT3 and fT4 concentrations was weak and this biochemical marker was relatively stable during all the experiment. In the other hand, in groups I and II, fT3 and fT4 concentrations presented a greater scattering and even marked fluctuations according to time were noticed. DEX induced a significant ($P <0.05$) decrease of serum fT4 concentration 24 h after the treatment initiation in comparison to initial values. Plasma levels decreased from 10.12 ± 0.95 to 8.04 ± 1.30 and from 10.56 ± 0.56 to 8.61 ± 1.45 for group I and group II respectively. Thereafter, serum fT4 undertake a sharp increase from 24th to 48th h and then gradual increase to 120th h after the beginning of the treatment; This significant increase ($P <0.01$) of the plasma concentrations observed in the treated animals remains between 48th and 120th following the beginning of the treatment. fT4 serum concentrations observed for group I and II at the 120th h exceeded both baseline and control values, the difference was significant ($P <0.05$). It should be noted that 24 h after the end of the treatment, fT4 serum concentrations decreased and reached baseline values.

More marked fT3 variations were observed in group I and II. An important and significant ($P <0.001$) drop was immediately observed within 6 h after the first injection of DEX. The values of fT3 decreased from 4.36 ± 0.22 to 2.77 ± 0.57 for group I and from 5.04 ± 0.32 to 2.92 ± 0.43 for group II respectively. In addition, the fT3 values obtained in group I continued to decrease until the 24th h, before increasing significantly ($P <0.05$) 48 h after the start of treatment. Serum fT3 levels in group II increased significantly ($P <0.05$) from the 6th to reach their maximum values (5.60 ± 1.76) 48 h after the start of treatment, exceeding therefore the baseline and the control values. It should be noted that the magnitude of the increase in fT3 plasma concentrations observed in group II was significantly greater than in group I ($P <0.05$). Thereafter, the serum fT3 values obtained in group I underwent a gradual and significant ($P <0.05$) rise until the 120th h after the beginning of the treatment, then reached baseline values and stabilized for the rest of the experiment. In addition, the fT3 plasma levels obtained in group II returned to baseline values earlier (72 h after initiation of treatment) and remained relatively stable until the end of the experiment.

Table 1: Temporal evolution of free thyroxin plasma concentrations (pmol/;)

Lots	48 h before	6 h	24 h	48 h	72 h	96 h	120 h	24 h after	6 d after	14 d after
control	10.29 ± 1.47	11.41 ± 1.00	10.45 ± 1.45	11.80 ± 1.03	10.77 ± 0.79	10.71 ± 1.09	10.74 ± 1.59	10.83 ± 1.81	12.01 ± 1.63	10.22 ± 2.17
Group I	10.12 ± 0.95	9.05 ± 1.02	8.04 ± 1.30	11.01 ± 1.23	12.28 ± 1.48	12.64 ± 1.10	12.95 ± 1.02	11.42 ± 1.41	10.68 ± 0.69	10.00 ± 1.96
Group II	10.56 ± 0.56	9.84 ± 0.92	8.61 ± 1.45	12.04 ± 2.01	12.13 ± 1.18	12.04 ± 1.04	12.59 ± 1.11	11.04 ± 0.69	10.64 ± 1.13	10.68 ± 1.26

Table 2: Temporal evolution of free triiodothyronine plasma concentrations (pmol/l)

Lots	48 h before	6 h	24 h	48 h	72 h	96 h	120 h	24 d after	6 d after	14 d after
Control	4.64 ± 0.55	3.75 ± 0.39	3.93 ± 0.63	4.30 ± 0.45	4.15 ± 0.61	3.64 ± 0.22	4.34 ± 0.91	4.36 ± 1.22	4.64 ± 0.87	4.08 ± 0.79
Group I	4.36 ± 0.22	2.77 ± 0.57	2.54 ± 0.64	3.73 ± 0.48	3.83 ± 0.60	4.19 ± 0.55	4.56 ± 0.59	4.30 ± 0.71	4.40 ± 0.23	4.27 ± 0.89
Group II	5.04 ± 0.32	2.92 ± 0.43	3.39 ± 0.97	5.60 ± 1.76	4.43 ± 1.13	4.40 ± 0.96	5.03 ± 0.51	4.73 ± 0.89	4.86 ± 1.25	4.62 ± 0.77

DISCUSSION

In the literature, one of the commonly admitted hypotheses about the effects of glucocorticoids on thyroid function is that they would cause functional hypothyroidism. In our experiment, we evaluated the effect of intramuscular injection of anti-inflammatory (1.52 mg/day for 6 days) and immunosuppressive (3.4 mg/day for 6 days) doses of DEX on free thyroid hormones plasma concentrations. The main finding in our trial is that the administration of DEX in IM caused a significant decrease ($P <0.05$) of fT4 and fT3 plasma levels within few hours after starting the treatment. Furthermore, no difference was observed between the two groups receiving DEX. Our findings are similar to those of Forhead et al. [13], who have found that T3 and T4 plasma concentrations decreased in pregnant ewes after injecting them with DEX at the rate of (2 × 12 mg/IM). In humans also, a decrease in T3 (In normal and a thyroid subjects) and T4 (in normal subjects) serum concentrations have been noticed following the administration of DEX at the rate of (2 mg/6 h for 48 h) [14]. In addition, topical (dermal and ototopical) DEX application in dogs for three weeks at therapeutic doses have severely suppressed serum thyroid hormones levels (T3 and T4), thus a secondary hypothyroidism could be provoked [12]. However, no significant changes in T3, T4 and rT3 plasma levels were observed in pregnant women at term, following administration of 8-16 mg Of DEX 3 to 48 h before an elective caesarean section [15].

In our experiment, the fall in TH plasma levels could be explained by an inhibitory effect of Dexamethasone on the hypothalamic-pituitary-thyroid axis. The administration of a high dose of DEX suppresses TSH and decreases the pituitary response to TRH in both humans and animals [4]. However the DEX suppressive effect on TSH secretion couldn't be confirmed in our experiment, because of the unavailability of the TSH assay.

In addition, the significant increase in fT4 and fT3 serum levels, observed 48 h after initiation of therapy (24 h after reaching their lowest values), suggests that low concentrations of thyroid hormones (TH) provoked a positive feedback on the release of pituitary TSH, leading to an

increase in their own secretion. This discards the hypothesis of the central effect of DEX or suggests that this effect is possibly only temporary. DEX modifies the secretion and peripheral metabolism of thyroid hormones [14]. The severe decrease in plasma concentrations of free TH observed in our trial could therefore be explained by the reduction in thyroid hormone secretion due to the reduction in lysosomal hydrolysis of the colloid, caused by the anti-inflammatory effect of GCs, which stabilizes the lysosomal membranes [8,9,11,15].

Thyroid hormones plasma concentrations are controlled by their bioavailability and clearance, it is possible that one of these two parameters may subsequently be altered [16]. GCs reduce human thyroid hormones by reducing serum TBG levels, or modifying their binding affinity for these hormones [17]. This hypothesis seems to be unlikely because, notwithstanding a decrease in TBG and consequently a decrease in total hormones, the free hormone concentration would have remained unchanged, but in our work the fT4 and fT3 observed variations were in favor of a significant reduction. In addition, the hormone released from TBG can be moved from the plasma to a large tissue sink, so it is the intrinsic plasma clearance that controls the free TH levels and probably could be the reason behind the depression of fT4 and fT3 plasma levels [18]. It should also be noted that the decrease in free TH induced by the administration of the anti-inflammatory dose (1.52 mg/day) was similar to that induced by the immunosuppressive dose (3.4 mg/day); No difference was detected comparing the two groups who have received the DEX treatment. The dose effect was therefore not shown in our study. This result is in contradiction with that obtained by Kurtdede et al. [9] where fT4 concentrations were more significantly reduced in dogs who received the immunosuppressive dose than in those who received the anti-inflammatory dose. It should be noted that in our study, the decrease of fT3 preceded that of fT4.

The lowest fT4 concentrations (8.036 ± 1.295 pmol/L for group I and 8.612 ± 1.453 pmol/L for group II) were observed 24 h after DEX injection, while those of fT3 (2.7680 ± 0.5739 pmol/L for group I and 2.9160 ± 0.4285 pmol/L for group II) were found a little earlier (6 h after the injection of DEX). This difference is probably due to the fact that 80% of the fT3 come from the peripheral deiodination of fT4 and only 20% comes from the thyroid [16,19], so the fT3 decline would be more drastic and more precocious than the fT4's. Finally, concerning the reversibility of the effects induced by DEX, described above, a return of fT4 to the initial values was demonstrated 24 h after the drug withdrawal; however, fT3 plasma levels returned to the baseline earlier (96 h after initiation of treatment).

These results suggest that the effects of DEX on thyroid function are transient. Our results are comparable to those obtained by Kurtdede et al. [9] who have found that the decrease in fT4, T4 and T3 levels caused by anti-inflammatory and immunosuppressive treatments with high doses of prednisolone was Reversible, and that 15 days after the end of both treatments, T4 and fT4 concentrations were similar to the control values. However, after using dermal and ototopical DEX, TH plasma levels, decreased and remained low seven days after the drug withdrawal [12]. Also, after administrating dermal DEX in horses, TH serum levels remained depressed 3 weeks after the drug withdrawal [20]. It should be noted that in all these studies glucocorticoids were used orally or topically. The difference or even the contradiction observed between the results obtained in the previous works compared with those obtained in our study could be explained by the fact that the effects of the glucocorticoids on the thyroid gland depend on the dose, the duration of the treatment, mode of administration and the type of synthetic glucocorticoids used [10].

CONCLUSION

Treatment with DEX causes a reduction in the plasma levels of the free thyroid hormones fT4 and fT3, which translates into functional hypothyroidism. Serum thyroid hormone concentrations returned to their initial values after the end of the treatment thus the functional hypothyroidism caused by DEX is only transient and therefore cannot have a significant impact on the health status of the animals.

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