Severe Thyrotoxicosis Does Not Accelerate 1α-Hydroxylation of 25-Hydroxyvitamin D3 in Dogs. Experimental Study

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ABSTRACT

Two healthy dogs weighing 18 kg and 13 kg each received an intravenous injection of 7 μg/kg 25-hydroxyvitamin D3 (25OHD3). Subsequently, they were blood-sampled in order to determine the plasma levels of 25(OH)D3 over 4-hourly time intervals and for a time period of 24 hours. After a period of 18 days since the last blood sampling, the animals were brought to a hyperthyroid state and the intravenous injection of 7 μg/kg 25OHD1 was repeated. Blood sampling was performed every 4 hours and over a time period of 24 hours in order to determine the levels of 25OHD1. The graphic plotting of plasma levels of 25OHD1 in the euthyroid state did not differ from that in the hyperthyroid state. This finding in dog animal experimentation is indicative that the increased levels of thyroid hormones did not affect the activity of CYP27B1 and CYP24A1 enzymes that are related to the catabolism of 25OHD3 over a minimum of 24 hours period.

Keywords: Thyrotoxicosis; Vitamin D; Dogs

1. Introduction

Thyrotoxicosis represents a morbid condition that gradually deprives the body of Vitamin D. In thyrotoxicosis, the plasma levels of 1,25-dihydroxyvitamin D (1,25(OH)2D) are depressed. Moreover, hydroxylation of Vitamin D on C25 position for the production of 25-hydroxyvitamin-D (25OHD) is accelerated resulting in low plasma levels of Vitamin D [1-3]. The accelerated hydroxylation of Vitamin D on C25 position in cases of thyrotoxicosis is due to the stimulation of liver microsomal enzymes (CYP27A1, CYP2J3, CYP2R1, CYP3A4) by the thyroid hormones. In addition, the pathogenesis of the attenuated levels of 1,25(OH)2D has not been fully understood. Parathormone, serum calcium and phosphorus, CYP enzymes (CYP24A1, CYP27B1) VDR, steroid and xenobiotic receptor (SXR) have all been reported as being involved in the pathogenesis of the “depressed” levels of 1,25(OH)2D in thyrotoxicosis [4].

In the following research study, we have attempted to investigate the influence of thyroxine on the CYP27B1 enzyme, which through hydroxylation of 25OHD leads to the production of 1,25(OH)2D.

2. Materials

The study involves two dogs weighing 18 kg (dog “W”) and 13 kg (dog “B”), respectively. These dogs initially were examined by a veterinary physician and were then placed in a specially designated area for experimentation (Vivarium) measuring 1 × 1 × 2 m with conditions of 20°C - 24°C temperature, good ventilation and lighting systems and with excellent hygiene conditions. The animals received standardised food and water (adlibidum). They were weighed daily and their pulse was monitored and ranged between 90 - 100/min. The animals remained in the designated areas for monitoring and for adaptation to this new environment and personnel for a time period of 9 days. Table 1 shows the biochemical findings in the blood of the animals.

3. Methods

The animals received an intravenous injection of 7 μg/kg 25OHD3 (approximately 5000 iu) and were subsequently
blood sampled every 4 hours and for a total time period of 24 hours. Eighteen days after the last blood sampling, the dogs were administered thyroxine at a dose of 0.5 mg/kg of body weight (IM-intramuscularly). Ten days later the animals were in a severe hyperthyroid state, as confirmed by physical examination (severe tachycardia that could not be clinically monitored, aggression, tremor, muscle weakness, constant barking, thirst, weight loss of 1 - 2 kg etc) and mainly by the thyroid function tests that showed low values prior to the start of the intervention and high values at the end of the study (comparisons made with human subject values). During the thyrotoxicosis phase and due to the severe hyperstimulation the animals received large doses of chlorpromazine, diazepam, nembutal and β-blockers, since the animals showed great resistance to these substances. At the hyperthyroid phase, the animals had an intravenous injection of 7 μg/kg 25OHD$_3$ and were blood sampled at 4-hourly intervals for a time period of 24 hours.

4. Results

Euthyroid phase: The intravenous infusion of 7 μg/kg of body weight 25OHD$_3$ to dog “W” gave the following values: minimum of 103.3 ng/ml (20th hour), maximum of 179.60 ng/ml (4th hour) and mean value of 123.9 ± 28.2. The intravenous infusion of 7 μg/kg of body weight to dog “B” gave the following values: minimum of 98.8 ng/ml (4th hour), maximum of 118.6 (20th hour) and mean value of 108.3 ± 6.7.

Hyperthyroid phase: The intravenous infusion of the same dose of 25OHD$_3$ per kg of body weight to dog “W” gave the following values: minimum of 109 ng/ml (20th hour), maximum of 150.5 ng/ml (12th hour) and mean value of 125 ± 16.4. The intravenous infusion to dog “B” gave the following values: minimum of 112.4 ng/ml (12th hour), maximum of 119.0 ng/ml (20th hour), and mean value of 116 ± 2.4. The values of 25OHD$_3$ in both animals and measured 4-hourly in an euthyroid state and in a hyperthyroid state are demonstrated in Table 2 and Figure 1. There was no statistically significant difference between them (p = NS). The thyroxine values at the euthyroid phase and the rest of the findings that are related to the thyroxine metabolism are displayed in Table 3. The health of the animals had completely recovered one month after the last blood sampling.

5. Discussion

The plasma levels of 25OHD$_3$ during the hyperthyroid phase of the animals showed no difference in comparison to those levels in the euthyroid phase. This indirectly shows that the values of 25OHD$_3$ were not deranged to the hydroxylated products (1,25(OH)$_2$D$_3$ and 24,25(OH)$_2$D$_3$) under the effect of the increased levels of thyroid hormones.

The thyrotoxicosis of the animals was indisputable as shown from the thyroid function tests (Table 3) and the clinical presentation of animals.

It is possible that the significantly raised levels of the...
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Table 1. Biochemical findings of dogs in the euthyroid phase (E) and the thyrotoxic phase (T).

|     | Na  | K   | Cl  | CO₂ | Prot | Ca  | P   | Uric acid | Cr. | Bilir. | LDH |
|-----|-----|-----|-----|-----|------|-----|-----|-----------|-----|--------|-----|
| WE  | 138 | 4.9 | 99  | 19.5| 6.7  | 9.3 | 2.8 | 5.5       | 0.9 | 0.8    | 288 |
| WT  | 144 | 4.1 | 113 | 11.0| 5.8  | 9.1 | 8.2 | 1.3       | 0.8 | 0.1    | 535 |
| BE  | 152 | 4.6 | 114 | 15.5| 5.9  | 10.4| 4.3 | 1.0       | 0.9 | 0.3    | 430 |
| BT  | 152 | 4.8 | 114 | 15.0| 5.4  | 9.9 | 9.3 | 1.5       | 1.1 | 0.3    | 433 |

Table 2. Mean values of 25OHD₃ of both animals on a 4-hourly basis in the euthyroid and hyperthyroid phase. There was no statistically significant difference between the values in the euthyroid and hyperthyroid phase.

| Euthyroid phase | 4 hours | 8  | 12  | 16  | 20  | 24  |
|-----------------|---------|----|-----|-----|-----|-----|
|                 | 139.2   | 119.9| 116.3| 106.9| 110.9| 117.0|
| ±57.1           | ±10.5   | ±5.5 | ±3.1 | ±10.8| ±7.0 |

| Hyperthyroid phase |
|--------------------|
| 126.5              |
| ±17.1              |

Table 3. Thyroid gland function values in the euthyroid phase (E) and the thyrotoxic phase (T).

|     | RiaT4 | RiaT3 | RT3U | γGT | ALP | Chol. |
|-----|-------|-------|------|-----|-----|-------|
|     | µg%   | ng%   | %    | U/L | mu/ml| mg%   |
| WE  | 1.0   | 50    | 61.0 | 1   | 52  | -     |
| WT  | >24   | >800  | 65.7 | 8   | 208 | 119   |
| BE  | 2.4   | 60    | 60.1 | 0   | 75  | 178   |
| BT  | >24   | >800  | 62.6 | 3   | 111 | 127   |

thyroid hormones in comparison to their levels during the euthyroid phase (2 µg%), and also the suppressive medication given during the hyperthyroid phase, may have affected in a negative way the enzyme activity or may have almost completely suppressed their function. The dose of 7 µg/kg 25OHD₃ (~3600 - 5000) is most likely excessive for animals weighing 13 and 18 kg. Nevertheless, it was taken into consideration that the plasma levels of 25OHD₃ in dogs has a wide variation ranging from 5 to 70 ng/ml depending on the respective reference range and also on the type of food being received [10]. This dose gave high plasma levels of 25OHD₃ in the animals both during the euthyroid and hyperthyroid phase. These levels never exceeded the toxic levels which for humans are defined as above the limit of 150, 206, 490, 690 and 468 ng/ml [11], and the serum calcium was similar in the euthyroid and hyperthyroid state.

Due to the lack of data about the physiology of vitamin D in dogs, it was not possible to evaluate the role of thyroid hormones on the metabolites and final products of vitamin D.

This study focused on the function of CYP27B1 cytochrome, which contains the 1α-hydroxylase enzyme which hydroxylates 25OHD₃ in the a-position.

6. Conclusions

The significantly increased levels of thyroid hormones did not affect the increased plasma levels of 25OHD₃ in the dogs that were tested 4-hourly and for a time period of 24 hours. These findings lead us to the following assumptions:

1) The excessive increase in the plasma levels of thyroid hormones inhibited the activity of the hydroxylating enzymes of 25OHD₃ in the dogs, in contrast to the stimulation of the liver microsomal enzymes (CYP27A1, CYP3A4, CYP2J3, CYP2R1 which in humans induce the rapid hydroxylation of vitamin D) in position 25.

2) It is necessary to allow for more time beyond 24 hours to have a more definite result.
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3) The significant increase in supply of 25OHD₃ to the CYP27B1 enzyme inhibited or slowed down the enzyme activity.

4) The thyroid hormones have no effect on the hydroxylating enzymes of 25OHD in dogs. FGF23-clotho reduces the blood levels of 1,25(OH)₂D₃ [12]. There is a lack of data in literature for the correlation of thyroxine and the axis of osteoblasts-osteocytes-PTH-FGF23-clotho and 1,25(OH)₂D₃.

7. Addendum

In 1633 Galileo Galilei quoted the historical phrase “Ep-pure si muove (still it moves, and yet it moves) when he was forced from “Holy Inquisition” to recant his belief that the earth moves around the sun (The event was first reported in English print in 1757 by Giuseppe Baretti) [13]. A minor yet similar event occurred to me as well. In my case, the “Holy Inquisition” was my research study that forced me to state that the increased levels of thyroid hormones in dogs do not influence the metabolism of 25OHD₃. On the contrary, I believe that the increased blood levels of thyroid hormones in the body accelerate the catabolism of vitamin D to give the end products of calcitroic acid and lactones [14]. In favour of this theory are: 1) the accelerated hydroxylation of Vitamin D on C25 position in thyrotoxicosis, 2) the depressed levels of 1,25(OH)₂D₃ again in thyrotoxicosis, 3) the increased 1,25(OH)₂D₃ blood levels in hypothyroidism [15]. I believe that a more detailed investigation in human subjects will clarify these issues in the future.

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