Influence of Cholecalciferol (Vitamin D3) on the Course of Experimental Systemic Lupus Erythematosus in F1 (NZBxW) Mice

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The course of systemic lupus erythematosus (SLE), an autoimmune disease, is markedly affected by hormones such as estrogen and prolactin. It is well known that heavy exposure to sunlight has deleterious effects on SLE, triggering episodes of the disease. Classical explanations for this occurrence suggest that UV radiation damages DNA, which becomes immunogenic, or induces exposure of the Ro antigen in keratinocytes. In recent years, it has been shown that vitamin D3 has important effects

on the immune system. Thus, we proposed an alternative hypothesis, suggesting that UV radiation, by promoting vitamin D3 synthesis, could be a factor aggravating the course of SLE after exposure to sunlight. To test this hypothesis, we injected F1(NZBxW) mice, which are prone to developing SLE, with vitamin D3, and we demonstrated a worsening of the histopathological findings in the kidney. *J. Clin. Lab. Anal.* 14:91–96, 2000. © 2000 Wiley-Liss, Inc.

Key words: lupus nephritis; vitamin D3; hormones; autoimmune disease; ultraviolet radiation

INTRODUCTION

Interactions between the immune and neuroendocrine systems have been demonstrated, with peptides from each system modulating each other's activities (1–3). Hormonal influences on the evolution of autoimmune diseases have been well established (4,5). The literature on systemic lupus erythematosus (SLE) is rich in examples of hormones such as estrogen, prolactin, and dehydroepiandrosterone influencing the outcome of the disease (4–8).

Calcitriol, the active form of vitamin D3, has been classically associated with the homeostatic mechanisms that maintain an adequate balance of calcium between blood and bone (9). Indeed, many authors have reported an important role of this hormone and its synthetic analogs in immune system function (10–12). In some situations, vitamin D3 has been used in therapeutic trials with good results (13).

It is well known that lupus patients heavily exposed to sunlight show a flare in their disease (14,15). Thus, the objective of the present study was to examine the possibility that vitamin D3 acting on the immune system may be responsible for worsening of the disease, providing new insights into the mechanisms associated with flares of the disease after exposure to sunlight.

MATERIALS AND METHODS

F1 (NZBxW) mice, 22 males and 20 females, were used as the experimental model. The animals were separated by sex and divided into three groups: Group I, injected with 10 µg cholecalciferol; Group II, injected with 3 µg cholecalciferol; and Group III, control injected with the vehicle solution without vitamin D3.

The vitamin D3 solutions were prepared as follows: 1.0 g of cholecalciferol (Solvay Duphar bv) was mixed with 9.0 g of dextrose and the final stock solution was maintained at 4°C. The 10-µg solution was prepared by dissolving 0.072 g of the stock solution in 0.5 ml of ethyl alcohol and adding 72.0 ml of distilled water, yielding a solution of 100 µg/ml (400 IU). The 3 µg solution was prepared by dissolving 0.0216 g of the stock solution in 0.5 ml of ethyl alcohol and adding 72.0 ml of distilled water, yielding a solution of 30 µg/100 ml (120 IU). Both the 3-µg and 10-µg solutions were fil-

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tered through 0.22 Millipore filters and stored at -20°C in 0.1 ml aliquots. The animals were injected intraperitoneally once a week for seven months starting at two months of age.

Blood was collected by venipuncture of the lacrimal plexus at 4, 5, 6, and 7 months during the experiment. The animals were then sacrificed by excess ether inhalation and the kidneys were removed for histopathological study.

Antinuclear antibodies were studied by the method described by Norins and Holmes (16). The kidneys were maintained in 10% formaldehyde for 24 hours, washed in distilled water for 4 hours and fixed in 70% alcohol. The material thus obtained was stained with hematoxylin-eosin and PAS.

The histopathological findings were analyzed by a semi-quantitative method, the Austin-NIH activity chronicity index of lupus nephritis (17,18).

The Kruskal-Wallis test was used for statistical analysis. When significant differences were detected, the Dunn test for multiple comparisons was used.(19).

RESULTS

Treatment with cholecaliferol led to a worsening of the histopathological findings in the kidneys of female F1 (NZBxW) mice. Table 1 presents the score obtained after 3 μg of vitamin D3 using the Austin index of lupus nephritis activity. It can be seen that the Austin index score was significantly higher in this group compared to control (Fig. 1).

The group of female mice injected with 10 μg cholecaliferol showed higher Austin index scores (Table 2) than the control group (Table 3), although the difference was not statistically significant (Fig. 1). In contrast, among male mice, despite some minimal histopathological differences in the group injected with 3 μg cholecaliferol, no significant differences were observed between the groups injected or not with vitamin D3 (Fig. 2).

Figures 3, 4, and 5 illustrate some histological aspects observed in female mice injected with vitamin D3 (Figs. 3 and 4) or not injected (Fig. 5).

DISCUSSION

The present data demonstrate that cholecaliferol, the precursor of the active form of vitamin D3, aggravates the his-

Fig. 1. Median score obtained from the Austin activity index for female F1 (NZBxW) mice injected with 10 μg vitamin D3, and for the control group. Data were analyzed statistically by nonparametric analysis of variance (Kruskal-Wallis) followed by multiple comparison by the Dunn test. Control ($n = 11$), 3 μg ($n = 5$), and 10 μg ($n = 6$); $P < 0.05$ compared to control.

topathological lesions of experimental lupus nephritis in female F1 (NZBxW) mice. Many animal models have shown that hormones can modulate the immune response. Thus, based on the response to hormones, it was proposed to classify autoimmune diseases into two groups (4): a group with a polyclonal response involving activation of B cells and the presence of circulating immunocomplexes, with systemic lupus erythematosus being a paradigm of this group, and a second group whose pathophysiological mechanisms predominantly involve activation of mechanisms of the cellular immune response.

SLE shows a worsening in its evolution in the presence of estrogen and prolactin, and an improvement with the use of androgen (5). DHEA, a hormone secreted by the adrenal gland, has beneficial effects on the course of SLE (8), which are explained by an increase in the activity of lymphocytes with a Th1 pattern.

There is much interest in the factors that control the cytokine profile of T-helper lymphocytes, and frequently the

TABLE 1. Histopathological scores of kidneys from female F1 (NZBxW) mice injected with 3 μg vitaminD3

Animal	EH	LI	SHD	Necr. ($\times 2$)	Cellular crescents ($\times 2$)	Int. Inf.	Σ
1	1	1	3	2	3	2	15
2	2	2	2	1	0	0	8
3	2	2	2	1	0	1	8
4	3	3	3	1	0	1	11
5	1	1	1	0	0	1	3

EH, endocapillary hypercellularity; LI, leucocyte infiltrate; SHD, subendothelial hyaline deposits; Necr, fibrinoid necrosis-caryorrhesis; Int. Inf., interstitial infiltrates.

TABLE 2. Histopathological scores of kidneys from female F1 (NZBxW) mice injected with 10 μg vitaminD3

Animal	EH	LI	SHD	Necr. ($\times 2$)	Cellular crescents ($\times 2$)	Int. Inf.	Σ
1	2	2	2	1	0	2	8
2	1	2	2	1	0	1	7
3	2	1	2	2	2	1	11
4	1	1	1	0	0	1	3
5	1	1	1	0	0	0	3
6	2	2	1	0	0	0	5

EH, endocapillary hypercellularity; LI, leucocyte infiltrate; SHD, subendothelial hyaline deposits; Necr, fibrinoid necrosis-caryorrhesis; Int. Inf., interstitial infiltrates.

TABLE 3. Histopathological scores of kidneys from female F1 (NZBxW) mice not injected with vitamin D3

Animal	EH	LI	SHD	Necr. (× 2)	Cellular crescents (× 2)	Int. Inf.	Σ
1	1	1	1	0	0	0	3
2	1	1	2	0	0	0	4
3	2	1	2	0	0	0	5
4	2	1	2	0	0	0	5
5	1	0	1	0	0	0	2
6	1	1	1	0	0	0	3
7	2	2	1	0	0	1	5
8	2	2	1	0	0	1	5
9	1	1	1	0	0	0	3
10	1	1	0	0	0	0	2
11	2	2	2	0	0	1	6

Σ is the score obtained and indicates the degree of aggressiveness of lupus nephritis according to the Austin index; median = 4.

EH, endocapillary hypercellularity; LI, leucocyte infiltrate; SHD, subendothelial hyaline deposits; Necr, fibrinoid necrosis-caryorrhexis; Int. Inf., interstitial infiltrates.

important influence of the endocrine system is largely ignored. Peripheral tissues are rich in enzymes that can convert inactive circulating prohormones into molecules with profound effects on T-cell function (20). DHEA circulates as an inactive prohormone and is activated peripherally. Lymphocytes express receptors for the hormone, which promotes a pattern of Th1 immune response (20). Similarly, cholecalciferol, a prohormone, also needs to be activated and its active form calcitriol has important functions in the immune system, but brings about responses with a Th2 pattern (21–23).

The conversion to an active form of vitamin D3 occurs in the kidney and placenta (9). In pathological states, the conversion to the active form occurs in cells of the monocyte lineage, as observed in the chronic inflammatory lesions of tuberculosis (24) and sarcoidosis (25,26). Conversion is also

observed in vitro in macrophages stimulated with γ -interferon (27).

Vitamin D3 receptors are expressed constitutively in monocytes and macrophages (10). B and T lymphocytes express the receptor 24 hours after activation (28). The receptor is also found in medullary thymocytes (4).

Calcitriol inhibits lymphocyte proliferation in vitro (29) at physiological doses, with a decrease in the production of IL-2 (22,30), but not in the expression of the IL-2 receptor (29). The inhibition of cellular proliferation is caused by a block in the passage from the G1-A to the G1-B phase of the cell cycle (29). The inhibition of proliferation is higher in CD 8+ lymphocytes (12). Vitamin D3 also affects the rate of γ -interferon and GM-CSF secretion (26).

A decrease in the in vitro production of IgG and IgM has been reported, and a doubt exists about whether this is caused by a direct action on B lymphocytes or indirectly as a consequence of the effect on T lymphocytes (11). More recently, vitamin D3 was shown to have a selective effect in vivo on the production of different isotypes of immunoglobulins, with an increase in the production of IgG1 and secretory IgA, and a decrease of IgG2, suggesting that its action does not involve B lymphocyte suppression, but rather modulation of immunoglobulin production (31).

Cells of the monocyte lineage are those most intensely affected by exposure to vitamin D3. In addition to the expression of vitamin D3 receptor in the constitutive form (28), there is an increase in the production of hydrogen peroxide under the influence of the hormone, in the capacity of killing microbes, in the expression of Fc receptor (24), in the production of cytokines such as TNF and IL-1 (30), and in the production of heat shock protein (4). In contrast, there is a decrease in the expression of surface molecules of MHC and in the production of γ -interferon (24).

As we have seen, vitamin D3 has many effects on the immune system response. Since SLE is a disease in which an alteration in function of many compartments of the immune system has been demonstrated, we conducted a survey of the literature for papers relating the disease to the hormone. We found few works relating experimental SLE to vitamin D3 (32,33). These authors used the hormone in trials in order to find an immunosuppressive therapeutic agent with disappointing results since they were unable to demonstrate any important alteration in the course of the disease.

In our work, we focus on the immunopathogenesis caused by the use of high doses of cholecalciferol as it would be expected to occur after exposure to sunlight under physiological conditions (34), and at non-nephrotoxic doses (35). We believe that vitamin D3 can be used as an immunosuppressive agent in some pathological states but this is not the case for SLE. Vitamin D3 acts as an immunomodulator, with its actions varying according to the doses used, the target cell population, and the hormonal milieu. Indeed, in our experimental model, treatment of male mice with

Fig. 2. Median score obtained from the Austin activity index for male F1 (NZBxW) mice injected with 3 μ g and 10 μ g vitamin D3, and for the control group. Data were analyzed statistically by nonparametric analysis of variance (Kruskal-Wallis) followed by multiple comparison by the Dunn test. Control (n = 10), 3 μ g (n = 5), and 10 μ g (n = 5). There were no significant differences between groups ($P < 0.05$).

Fig. 3. Light micrograph of a glomerulus showing global endocapillary hypercellularity (H & E, $\times 250$).

Fig. 4. Light micrograph of lupus nephritis. A discrete interstitial inflammatory infiltrate and fibrinoid necrosis of arterioles can be observed. (H & E, $\times 250$).

Fig. 5. Light micrograph of renal parenchima showing minimal glomerular endocapillary hypercellularity(H & E, × 250).

cholecalciferol had no effect on the course of the disease (Fig. 2), whereas a deleterious action on kidney histology was evident in females (Fig. 1).

Many of the actions of vitamin D3 on macrophages, lymphocytes, or on the pattern of cytokine secretion could explain the worsening of the disease observed in our model. Daynes (31) showed that vitamin D3 altered the isotypes and subclasses of immunoglobulin production in vivo and a possible explanation for our results is that changes in the immunoglobulin produced, altering the immunocomplexes formed, could alter their pathogenicity and consequently the course of the disease.

Recently, many authors have demonstrated a role for macrophages in the pathogenesis of SLE (36–38). In the kidney, mesangial proliferation is a histological feature almost universal in lupus nephritis. Another possible explanation of our findings is that vitamin D3 could act on mesangial cells, which are part of the mononuclear phagocytic system, promoting activation of these cells, and consequently activation of the disease.

The pattern of cytokines found in SLE is Th2 (39). In experimental models, diseases with a Th1 pattern of cytokines, like experimental allergic encephalomyelitis and autoimmune diabetes mellitus could be prevented by the use of vitamin D3 (40,41). We think that the hypothesis that the hormone can drive cytokine secretion is reasonable, with an increase in the Th2 pattern and a worsening of the course of the disease occurring in the present study.

Finally, all the above hypothetical events can occur together, and we are searching for an explanation for the histopathological findings in our model. We believe that a comprehensive study of neuroendocrine influences in autoimmune diseases is of capital importance for the study of pathogenesis in these pathologies.

The results reported here should be considered within the context of this experimental model, F1 (NZB×W), which offers the possibility of new insights into the neuroendocrine modulation of autoimmune diseases.

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