Glucocorticosteroid dependent decrease in the activity of calcineurin in the peripheral blood mononuclear cells of patients with systemic lupus erythematous

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Abstract

Objectives—To compare the activity of calcineurin in the peripheral blood mononuclear cells (PBMC) of 32 patients with systemic lupus erythematosus (SLE) and 35 healthy controls.

Methods—The activity of calcineurin was assayed in the supernatants of sonicated mononuclear cells. On the other hand, the activity of calcineurin was reduced in patients with SLE taking GCS, correlating negatively with the dose of GCS. In the supernatants of PBMC by phorbol ester and calcium ionophore decreased the calcineurin activity both in patients with SLE and in healthy controls. GCS could also reduce calcineurin activity in the mononuclear cells of healthy subjects in vitro.

Results—There was no significant difference in the calcineurin activity of patients with SLE not taking glucocorticosteroids (GCS) compared with the healthy controls. On the other hand, the activity of calcineurin was reduced in patients with SLE taking GCS, correlating negatively with the dose of GCS. The stimulation of PBMC by phorbol ester and calcium ionophore decreased the calcineurin activity both in patients with SLE and in healthy controls. GCS could also reduce calcineurin activity in the mononuclear cells of healthy subjects in vitro.

Conclusions—In patients with SLE the decrease in the calcineurin activity of PBMC depended on the dose of GCS used for treatment, and it was not a disease specific alteration. The higher the dose of GCS, the greater the inhibition of calcineurin activity. The reduction of calcineurin activity is a new element in the immunosuppressive effects of GCS during the treatment of patients with SLE.

(Foreign language: English)

In this study we show that in patients with SLE not taking any glucocorticosteroids (GCS) the calcineurin activity of peripheral blood mononuclear cells (PBMC) does not differ from that of healthy controls. On the other hand, in patients with SLE taking various doses of GCS, decreased calcineurin activity can be measured. The higher the concentration of GCS, the greater the decrease in calcineurin activity. The in vitro stimulation of PBMC by phorbol ester and Ca²⁺ ionophore (A23187) results in a pronounced decrease in the calcineurin activity of cells derived from patients with SLE or from healthy controls. GCS can reduce the calcineurin activity also in the mononuclear cells of healthy subjects in vitro.

Patients and methods

PATIENTS, CLINICAL DATA, AND CONTROL GROUP

The SLE study group comprised 32 patients, 29 women and three men, with a mean age of 36 years (range 18–68). The mean SLE disease activity index (SLEDAI) was 6.1 (range 2–20). The average dose of GCS (methylprednisolone or equivalent of prednisolone) taken by the patients was 9.8 mg/day (range 0–32). These patients were free from any cytostatic drugs. The average duration of the disease was 8.5 years (range 0.5–35). Three subgroups of these patients were studied: (a) seven patients (six women, one man) not taking any GCS, median age 41 years (range 28–59), mean SLEDAI 2.43; (b) six patients (five women, one man) taking 8 mg of GCS/day, median age 39 years (range 19–52), mean SLEDAI 4.4; (c) six patients (five women, one man) taking 16 mg of GCS/day, median age 36 years (range 18–55), mean SLEDAI 11.5. Thirty five healthy white subjects (32 women, three men), median age 36 years (range 20–52), served as controls. Approval was given through the institutional review board, and informed consent was obtained from all participants. All patients with SLE fulfilled the diagnostic criteria of the American College of Rheumatology with at least four of the revised criteria for the diagnosis of SLE. Patients with a SLEDAI score <3 were considered to have inactive disease.
Calcineurin in PBMC of patients with SLE

**Results**

During the first phase of the experiment we compared the activity of calcineurin in the PBMC of patients with SLE and in healthy controls. As the calcineurin activities obtained from the patients with SLE were diverse, the daily doses of GCS were determined in the patients after 72 hours in the absence and presence of 10 μM prednisolone sodium succinate (Diadreson, Organon, The Netherlands) in RPMI medium with fetal calf serum (10%).

**CULTURING OF PBMC IN THE PRESENCE OF PREDNISOLONE SODIUM SUCCE NATIVE**

PBMC of five healthy controls were cultured for 72 hours in the absence and presence of 10 μM prednisolone sodium succinate (Diadreson, Organon, The Netherlands) in RPMI medium with fetal calf serum (10%).

**STATISTICAL ANALYSIS**

Statistical means and SD values were calculated to compare the activity of calcineurin in the patients with SLE and in the healthy controls. The statistical significance of the differences was evaluated by Student's paired t-test. During measurements of the in vitro effects of GCS on calcineurin activity, each value was calculated as the mean of the data from five healthy control subjects. In these experiments the statistical significance was calculated by Student's paired t-test. The correlation coefficient between the calcineurin activities and the doses of GCS was determined in the patients with SLE. In these studies the calcineurin activities of the individual patients were expressed as a percentage of the activities measured in the healthy controls, which were considered to be 100%.

**Negative correlation between the basal activities of calcineurin in the peripheral blood mononuclear cell (PBMC) of patients with SLE and the daily doses of glucocorticosteroids (GCS) used for treatment.**
with SLE was calculated. It was found to be highly significant: \( r = -0.52 \) (\( p < 0.001 \)). The inhibition of calcineurin activity in the PBMC of patients with SLE may be a new factor in the immunosuppression caused by GCS. There was no significant difference in the calcineurin activities of seven GCS-free patients with SLE and those of 35 healthy controls (fig 1).

CALCINEURIN ACTIVITIES IN THE PBMC OF PATIENTS WITH SLE WITH OR WITHOUT GCS TREATMENT AND IN HEALTHY CONTROLS

In four groups of subjects the calcineurin activities of PBMC were assayed and compared. The cells were either non-stimulated or stimulated by PMA and Ca\(^{2+}\) ionophore. In the non-stimulated cells of six patients with SLE taking 32 mg of GCS/day, there was a significant decrease in the calcineurin activity compared with the value of six healthy controls (9.7 mU/mg vs 15.1 mU/mg, \( p < 0.05 \)). In the seven GCS-free patients or in the six patients taking 8 mg of GCS/day, the changes were not significant compared with the values of the healthy controls (14.6 mU/mg and 12.8 mU/mg vs 15.1 mU/mg). In the cells stimulated by PMA and Ca\(^{2+}\) ionophore for four hours there was a marked decrease in the calcineurin activities of all groups compared with their...

![Graph showing calcineurin activities](image-url)
Calcineurin in PBMC of patients with SLE

The goal of these experiments was to measure the activity of calcineurin in the PBMC of patients with SLE. The major results are as follows. Firstly, there is no difference in the calcineurin activities of PBMC in the GCS-free cultures of the next two days: 24 and 48 hours, respectively (p<0.001). A similar tendency was seen in the cells treated with PRED. However, the calcineurin activity increased significantly from the value at 24 hours (6.4 mU/mg) to 14.2 mU/mg and 12.9 mU/mg at 48 and 72 hours, respectively (p<0.001 and p<0.01). The activity of calcineurin increased even further in the PBMC of healthy controls and patients with SLE treated with or without 10−6 M prednisolone sodium succinate (PRED) for 72 hours. This non-toxic dose of the drug had insufficiently inhibited calcineurin (p<0.001), whereas a slight but non-significant decrease in calcineurin activity was seen in the control cells (15.1 mU/mg).

There was no significant difference in the proportion of the various subsets of PBMC in the patients with SLE and the controls. PRED was chosen for the in vitro experiments because the derivatives of prednisolone were mostly used also for the treatment of the patients with SLE. PRED was judged not toxic in these studies, according to the lactate dehydrogenase measurements. It is known that all GCS have both genomic and non-genomic membrane effects. According to these reports, the 32 mg/day dose of GCS in our patients and the effect on calcineurin activity. We found no decrease in the amount of calcineurin detected by immunoblotting (data not shown) either in the cells of patients or in the cells of healthy controls treated with these doses of GCS, suggesting that the GCS-induced inhibition of calcineurin activity was independent of the synthesis of the enzyme. However, in these cells an increased rate of apoptosis was determined at 24 and 48 hours of culturing compared with the GCS-free cells (data are not shown). This observation is in accordance with an earlier observation that the rate of apoptosis is increased in the T cells of patients with SLE treated with GCS. Therefore, we suppose that calcineurin, a newly identified endogenous inhibitor of calcineurin, might possibly be one of the key molecules playing a part in this phenomenon, coupling the processes of T cell receptor regulation, apoptosis, and reduction of calcineurin activity in the PBMC. Furthermore, calcineurin plays a part in the reduced calcineurin activity seen in the cells stimulated by phorbol ester and Ca2+ ionophore in our experiments.

Our results confirmed the observation of Rider et al., who found no difference in the amounts of calcineurin mRNA in patients with SLE and healthy controls. According to our data this similarity was also reflected in the calcineurin activities of the PBMC of patients with SLE and healthy subjects. The significantly decreased calcineurin activity of PBMC measured in patients with SLE treated with 32 mg GCS/day, however, is a new observation. In addition, a significant negative correlation was found between the doses of GCS used in the treatment of patients and SLE.

Calcineurin in PBMC of patients with SLE after 24 hours: 16.8 mU/mg (PRED) for 72 hours. This non-toxic dose of GCS can be regarded as “T cell-rich suspensions.”

IN VITRO EFFECT OF PREDNISOLONE SODIUM SUCCINATE ON THE ACTIVITY OF CALCINEURIN IN PBMC OF HEALTHY SUBJECTS

To verify the inhibitory effect of GCS on calcineurin activity, the mononuclear cells of healthy controls were cultured with or without 10−6 M prednisolone sodium succinate (PRED) for 72 hours. This non-toxic dose of the drug had significantly inhibited calcineurin activity at 24 hours (16.8 mU/mg ± 6.4 mU/mg) (p<0.001), whereas a slight but non-significant decrease in calcineurin activity was seen in the control cells (15.1 mU/mg ± 16.6 mU/mg). The activity of calcineurin increased even further in the GCS-free cultures over the next two days: 26.7 and 21.6 mU/mg at 48 and 72 hours, respectively (p<0.001 and p<0.01). A similar tendency was seen in the cells treated with PRED.

Calcineurin is a Ca+2 dependent enzyme, and, therefore, the inhibition of Ca+2 influx by GCS may have an important role in the GCS-dependent decrease of calcineurin activity. This fact may partly explain the earlier finding that GCS inhibit the calcineurin-dependent activation of IL2 and IL4 genes. However, the inhibition of calcineurin by GCS is not a phenomenon unique to calcineurin, as it can be induced also in a dose dependent manner in the PBMC of healthy subjects in vitro.
The mononuclear cells of healthy controls cultured in vitro showed rather different patterns of calcineurin activity in the untreated and PRED treated series (fig 3). The gradually increasing calcineurin activities of control cultures could be attributed to the production of several cytokines (for example, TNF or IL1) derived from the mononuclear cells attached to the surface of plastic dishes used for the culturing. By increasing the intracellular levels of Ca2+, TNF and IL1 could significantly increase the calcineurin activity of cells cultured for longer than 24 hours. The significant inhibition of calcineurin seen in the cells cultured in the presence of PRED for 24 hours was described recently. However, the recovery of calcineurin activity in the PRED treated cells from its reduced level at 24 hours may be explained by the appearance of the cytokines (TNF and IL1) during the second day of culturing (48 hours). As PRED can also inhibit the cytokine production of cultured cells activated by the plastic surface, the calcineurin activity increased by the effect of cytokines could never reach the values seen in the GCS-free counterparts of these cells.

Our data show that the decreased calcineurin activity induced by high doses of GCS may represent a new element in the immunosuppressive treatment of patients with SLE.

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