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

S Sipka, K Szucs, S Szántó, I Kovács, G Lakos, E Kiss, P Antal-Szalmás, G Szegedi, P Gergely

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. 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.

Patients and methods

PATIENTS, CLINICAL DATA, AND CONTROL GROUP
The SLE study group comprised 32 patients, 29 women and three men, with a median age of 36 years (range 18–68). The mean SLE disease activity index (SLEDAI) was 4.1 (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 39 years (range 18–55), mean SLEDAI 4.4; (b) six patients (five women, one man) taking 8 mg of GCS/day, median age 39 years (range 19–52), 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

Calcineurin activity (%)
0 10 20 30 40 50 60 70 80 90

Healthy controls
Patients with SLE

Figure 1 Negative correlation between the basal activities of calcineurin in the peripheral blood mononuclear cells (PBMC) of patients with SLE and the doses of glucocorticosteroids (GCS) used for treatment. The coefficient of correlation between the basal activities of calcineurin in the PBMC of patients with SLE and in healthy controls was calculated by Student's paired t-test. The correlation coefficient was calculated as the difference between 

Calcineurin activity (%)

Dose of glucocorticosteroids (mg/day)

www.annrheumdis.com

et al.
with slight modifications. The assay mixture (30 µl) containing 50 mM Tris-HCl (pH 7.0), 0.3 mM dithiothreitol, 0.2 mM CaCl2, 1 mM MnCl2, 0.04 mg/ml calmodulin or 2 mM EGTA, an appropriate amount of extract (0.5–1.5 mg/ml protein), and 32P labelled protein phosphatase inhibitor 1 (12–25 000 cpm/reaction mixture) was incubated at 30°C for 10 minutes. All mixtures contained 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10 µg/ml trypsin inhibitor, 1 mM PMSF, 5 mM benzamidine as protease inhibitors, and 20 nM okadaic acid as a protein phosphatase 2A inhibitor. The activity of protein phosphatase 1 was not assayed under the conditions given above, because 200 nM of okadaic acid did not result in any inhibition. The reaction was terminated by the addition of 100 µl of 10% trichloroacetic acid. The supernatants were collected and stored in aliquots at −70°C. The activity of calcineurin was calculated as the mean of the data obtained from five healthy control subjects. In these experiments the statistical significance was calculated 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 obtained 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%.

CULTURING OF PBMC IN THE PRESENCE OF PREDNISOLONE SODIUM SUCCESSATE

PBMC of five healthy controls were cultured for 72 hours in the absence and presence of 10−7 M prednisolone sodium succinate (Diadreson-F-Aquosum, 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 unpaired 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%.

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 taken by the subjects were considered when the data were analysed.

NEGATIVE CORRELATION BETWEEN THE BASAL ACTIVITIES OF CALCINEURIN IN PBMC OF PATIENTS WITH SLE AND THE DOSES OF GCS USED FOR THE TREATMENT

The correlation between the basal calcineurin activities in the PBMC and the daily doses of GCS (0–32 mg/day) taken by the 32 patients...
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 Ca²⁺ 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 Ca²⁺ ionophore for four hours there was a marked decrease in the calcineurin activities of all groups compared with their

**Figure 2** Calcineurin activities in the peripheral blood mononuclear cells (PBMC) of patients with systemic lupus erythematosus (SLE) with or without glucocorticosteroid (GCS) treatment and in healthy controls. Calcineurin activities were assayed in the supernatants of non-stimulated and stimulated PBMC as described in “Patients and methods.” The statistical significance of the differences was calculated by Student’s unpaired t test. Asterisk denotes significant difference compared with the controls.

**Figure 3** In vitro effect of prednisolone sodium succinate (PRED) on the activity of calcineurin in peripheral blood mononuclear cells (PBMC) of healthy subjects. PBMC of five healthy controls were cultured for 72 hours in the absence and presence of 10⁻⁴ M PRED. Calcineurin activities were determined and the statistical analysis was carried out as described in “Patients and methods.” Asterisks denote significant differences compared with the respective controls. For the calculation of statistical significance Student’s paired t test was used.
Calcineurin in PBMC of patients with SLE

non-stimulated counterparts: 5.2, 4.9, 4.5, and 3.8 mU/mg; v = 14.1, 14.6, 12.8, and 9.7 mU/mg (p=0.001). The differences between the calcineurin activities of the stimulated cells in the four groups were not significant, but the changes showed a pattern similar to those found in the non-stimulated cells—the highest activity was measured in the healthy controls, the lowest in the patients receiving 32 mg of GCS/day (fig 2). In addition, we found no significant differences in the calcineurin activities of men or women with SLE taking or not taking GCS, but each group contained only one man (data not shown).

**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 10−4 M prednisolone sodium succinate (PRED) for 72 hours. This non-toxic dose of the drug had significantly inhibited calcineurin after 24 hours: 16.8 mU/mg (PRED) for 72 hours. This non-toxic dose of cytometry, and as it was found that 69.4% of subsets of PBMC were characterised by flow of various purified subsets of the cells. As the phopenic it would have been di number of cells. As patients with SLE are lym-...
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 Ca++, 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 from its reduced level at 24 hours.

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.

The authors thank Mrs Julia Hunyadi for expert technical assistance.


