Dyslipidemia can reduce the immunosuppressive effects of cyclosporine

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Abstract

Aims: Dyslipidemia is a significant risk factor for the development of atherosclerotic disease and of chronic allograft rejection. Few data are available on the effects of dyslipidemia on the immunosuppressive action of immunosuppressive agents. We investigate the in vitro effects of lipids solution on the immunosuppressive action of cyclosporine (CsA).

Methods: Peripheral blood mononuclear cells (PBMC) were PHA or OKT3 activated in vitro with/without different concentrations of Intralipid solution (INT, range 0.5% to 15%). CsA inhibition of activation was measured after a 3 day incubation, by adding H3-thimidine. The intracellular concentration of CsA was measured by radioimmunoassay and related to the CsA inhibitory effects.

Results: Increasing INT concentration in the medium, CsA inhibition of PBMC activation by PHA or OKT3 was reduced from 72±13% to 8±2% and from 80±10% to 18±3%, respectively. A significant reduction of the intracellular CsA concentration was also evident with increasing INT concentrations and was related to the inhibitory activity of CsA.

Conclusions: These results suggest that dyslipidemia may reduce the availability of intracellular CsA concentration to inhibit the immune activation process and may explain the relationship between dyslipidemia and chronic allograft loss.

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1. Introduction

Dyslipidemia is an important complication of kidney transplantation, affecting almost 70% of recipients [1]. Several studies have suggested that hyperlipidemia may play a role in chronic allograft nephropathy [2,3]. Dimény et al. have shown that pretransplant lipid levels correlated with posttransplant acute and chronic renal allograft rejection [4]. Ingulli et al. showed that severe hypercholesterolemia inhibits CsA activity in nephrotic syndrome [5]. More recently, Roodnat et al. reported on the relationship between high serum cholesterol levels and clinical outcome of kidney-transplant patients, showing that serum cholesterol levels have an independent influence on the graft and patient survival [6]. While dyslipidemia was advocated as a specific risk factor for the development of chronic allograft nephropathy [7]. Better graft survival was reported in transplant patients on lipid lowering drugs [8–10]. Cyclosporine is a lipophylic drug, and the presence of dyslipidemia may alter the drug’s distribution. As a consequence the intracellular CsA concentration may be reduced, as well as its immunosuppressive activities. The aim of this study was to investigate the in vitro effects of lipids on the CsA inhibition of mono and polyclonal lymphocyte activation.

2. Methods

2.1. Cell activation

Human peripheral blood mononuclear cells (PBMC) were obtained from peripheral blood of healthy donors by density-gradient centrifugation (MSL, specific density, 1.077 g/ml; Labtek, Eurobio, France). After three washings in HBSS (Labtek, Eurobio, France) cells were resuspended in culture medium RPMI 1640 supplemented with 2 mM l-glutamine, 100 U/ml penicillin/streptomycin.
and 5% FCS (Gibco, Grand Island, NY, USA) at 3 × 10^6 cells/ml. PBMC (1.5 × 10^7 /well) were stimulated with phytohemagglutinin-P (PHA; Difco Laboratories, Detroit, Mich, USA) at final concentration of 30 ng/ml, or with OKT3 monoclonal antibody (ORTHO, Raritan, NJ; 10 ng/ml) in 96 well round-bottomed microculture plates (Microtest III, Falcon; Becton and Dickinson, Mountain View, Calif., USA). CsA (100 ng/ml, final concentration) was added at the beginning of the culture period. Different concentrations of lipid solution (Intralipid 20% solution, Fresenius Kabi, Italy) were added to the culture medium at the beginning of the period. Different concentrations of lipid solution (Intralipid 20% solution, Fresenius Kabi, Italy) were added to the culture medium at the beginning of the cell culture (0.5%, 1%, 5%, 10%, 15% and 20%). Intralipid 20% solution (INT) contains 200 g soy lipids, 12 g egg lecithin, 22.5 g glycerol in 1 l of water. Cells were incubated for a period of 3 days at 37 °C with 5% CO2, 1% FCS (Gibco, Grand Island, NY, USA) at 3×10^6 cells/ml. PBMC (1.5×10^4/well) were stimulated with phytohemoagglutinin-P (PHA; Difco Laboratories, Detroit, Mich, USA). Cells were incubated for a period of 3 days at 37 °C with 5% CO2, 1 μCi (3H) Tdr (Amersham, Arlington Heights, Ill, USA) was added to each individual well 16–18 h before harvesting, on day 3. Cells were then harvested using a semi-automatic cell harvester (Mac III M, Tomtec 96, Orange, CT, USA) and the degree of thymidine incorporation was measured using a 1450 Microbeta Trilux liquid scintillation counter (Wallac, Inc., Gaithersburg, MD).

The percent of proliferation was expressed as Stimulation Index (SI):

$$SI = \frac{cpm \text{ activated cells}}{cpm \text{ resting cells}}$$

The effects of INT on the inhibitory capability of CsA was reported as the percent of inhibition using the formula:

$$\% \text{ inhibition} = \left(1 - \frac{cpm \text{ activated cells} + CsA + INT}{cpm \text{ activated cells} + CsA}\right) \times 100$$

2.2. Intracellular CsA concentration

At the end of the incubation period, the intracellular CsA concentration was measured, together with the CsA concentration in the supernatant. Briefly, cells in the presence of their supernatants were gently scrapped from each well of the incubation plate, then were centrifuged at 1200 rpm at 4 °C for 20 min. Cells were washed three times with a solution of HBSS. The pellets obtained were resuspended in the test tubes with 0.5 ml of 99.8% solution of methanol. CsA levels were determined by specific, commercially available radioimmunoassay (RIA) kit (Cyclo-Trac® SP-whole blood, DiaSorin, Stillwater, MN, USA).

2.3. Statistical analysis

All data are reported as mean±standard deviation (SD) of triplicate experiments, Student’s t test was applied for statistical analysis. P values<0.05 were considered statistically significant.

3. Results

PBMC were activated with PHA or OKT3 in the presence or absence of different concentrations of Intralipid (INT) solution: 0.5%, 1%, 5%, 10% and 15%. In a dose/response analysis the addition of different concentration of INT at the beginning of cell culture time did not significantly change the activation process of PBMC. Detailed data are presented in Table 1. INT concentrations over 15% showed toxic effect on PBMC and were not used in the experiments evaluating the CsA inhibitory activity.

When increasing concentrations of INT were added to PBMC activated with mitogens in the presence of 100 ng/ml CsA, a significant reduction of the inhibitory effects of CsA was noted. The percentage of inhibition of CsA was progressively reduced from 72±9 to 8±2 (P<0.0001) for PHA and from 80±10 to 18±3 (P<0.0001) for OKT3, with increasing INT concentrations (Fig. 1). To explain these results we measured the intracellular CsA concentration in PBMC from resting PBMC cultured with CsA (100 ng/ml). Values were mean intra-cellular CsA concentration±SD (n=3). * P≤0.005.

4. Discussion

This study shows that increasing concentration of lipids in the cell culture medium significantly reduce the intracellular concentration of CsA and the inhibitory effects of CsA. Chronic allograft nephropathy and cardiovascular diseases are the most frequent cause of renal transplant failure, accounting for 50–80% of graft losses [11,12]. CAN is characterized by a slow
but constant loss of graft function, with histological features of atherosclerotic lesions, glomerular sclerosis, interstitial fibrosis and tubular atrophy. Many immune or nonimmune risk factors have been recognized for CAN development: among these is dyslipidemia. The relationship between hyperlipidemia and immune events was first suggested by observational studies in renal transplant patients where correlations between plasma lipid levels and both acute and chronic rejection were reported [12–14]. Potential causes of hyperlipidemia in transplant recipients include diet, genetic predisposition, obesity and immunosuppressive therapy. Immunosuppressive regimens, particularly corticosteroids, rapamycin and cyclosporine, are known to lead to dyslipidemia in transplant recipients [15–18]. Kuster et al. [19] found cyclosporine blood levels significantly correlated to total plasma cholesterol, low-density lipoprotein cholesterol, apolipoprotein B and the cholesterol/high-density lipoprotein cholesterol ratio. Because addition of the INT solution in the cell culture showed no significant effect on mitogens PBMC activation, the inhibitory effect on CsA immunosuppressive activity may depend upon the reduced bioavailability of CsA in the presence of lipids in the medium. Consequently, the intracellular concentration of CsA was significantly lower in the presence of lipids. CsA is suggested to be incorporated into peripheral blood lymphocytes through low-density lipoprotein-(LDL) receptors on the cell surface such as LDL-bounded CsA complex. These findings could explain the CsA efficacy attenuation through an intervention of CsA uptake into lymphocytes via LDL-receptor down regulation. Ingulli et al. showed that severe hypercholesterolemia inhibits CsA activity in nephrotic syndrome [20], so high lipid levels may be associated with decreased CsA immunosuppressive activity and play a role in chronic allograft nephropathy [21]. The results of this study suggest that dyslipidemia may reduce the availability of CsA and interfere with its capability to inhibit the immune activation process.

References