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Does the switch from Sandimmun to Sandimmun Neoral reduce patient need for Phenihydine?

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Abstract In patients receiving cyclosporine A (CyA) – based immunosuppressive therapy, Ca^{2+} channel blockers (CCBs) prevent the development of CyA – related nephrotoxicity in which increased Ca^{2+} content plays an important role. We evaluated the dynamics of the intracellular (erythrocytes) and extracellular (plasma) Ca^{2+} levels and the influence of the CCB, Phenihydine, on this process during the conversion from Sandimmun (S) to Sandimmun Neoral (SN). Forty-two patients were enrolled. The conversion from S to SN normalized the elevated CA^{2+} level of erythrocytes in groups with Phenihydine ($n = 20$) and without Phenyhidine ($n = 12$) 4 weeks after the switch ($P < 0.05$); this level remained stable until the end of study. Therefore we suggest that the switch from S to SN is effective in reducing elevated intracellular Ca^{2+} levels. The decrease of Ca^{2+} content in erythrocytes was similar in all groups switched to SN (with or without Phenihydine). The last effect should be an important argument to focus the further long-term investigations on the ability of CCBs to act as cytoprotective and nephroprotective agents during immunosuppressive protocols with the new microemulsion formulation of CyA.

Key words Kidney transplantation · Immunosuppression · CyA formulations · Calcium channel blockers · Intracellular calcium

Introduction

An increase in intracellular calcium (iCa^{2+}) level occurs as the final common pathway of cell degeneration and death in response to many pathogenic factors, including low temperature, metabolic poisons, and pharmacological agents [5]. During cyclosporine A (CyA) – based immunosuppressive therapy, iCa^{2+} has been found to be elevated in several cell types, including platelets, red blood cells (RBC), and smooth muscle cells [4, 8]. The messenger role of Ca^{2+} requires its maintenance within cells at very low (submicromolar) ionic concentrations. This transcellular gradient is maintained by the reversible complexing of Ca^{2+} to specific proteins. iCa^{2+} can increase through an enhanced influx, release from intracellular stores, or a reduced efflux of Ca^{2+} from the cell [7].

The nephrotoxicity of CyA appears to be due to a vasoconstrictive effect, to a great extent related to an increased iCa^{2+} concentration [12]. At the same time, application of Ca^{2+} channel blockers (CCB) to patients who have undergone renal [9] and heart [3] transplantation and who have received triple immunosuppression including CyA, could prevent the development of nephrotoxicity symptoms. A new microemulsion formulation of CyA, Sandimmun Neoral (SN), possesses less nephrotoxicity than does Sandimmun (S) [2]. This was achieved by changing the pharmacodynamics of the preparation, which led to extension of the therapeutic window between that drug concentration necessary for clinical immunosuppression and that which produces nephrotoxicity [11].

The most convenient model for the study of intracellular levels of mineral elements are erythrocytes, espe-

Table 1 Dynamics of intracellular calcium concentration *(iCa^{2+})* after switching from Sandimmun *(S)* to Sandimmum Neoral *(SN)*

iCa^{2+} (mmol/l packed cells)	Before	Six weeks after switching from S to SN	Twelve weeks after switching from S to SN
Group 1	0.037 ± 0.002*	0.029 ± 0.003	0.025 ± 0.002**
Group 2	0.031 ± 0.001*	0.024 ± 0.002	0.022 ± 0.001***
Group 3	0.033 ± 0.002*	0.026 ± 0.001	0.023 ± 0.001**
Group 4 (control)	0.034 ± 0.001	0.03 ± 0.002	0.029 ± 0.002
Healthy volunteers	0.021 ± 0.001		

* $P < 0.01$ versus healthy volunteers, ** $P < 0.03$ versus start, *** $P < 0.05$ versus start

cially as these cells contain CyA-binding protein [1]. This study was designed to evaluate the dynamics of the intracellular (RBC) and extracellular (plasma) Ca^{2+} levels and the influence of the dihydropyridine group CCB, Phenihydine, on this process during the conversion from S to SN.

Materials and methods

Four groups of patients with stable allograft function were investigated. All patients were on triple immunosuppressive therapy with a daily dose of 3.9 ± 1.2 mg/kg CyA, 77.3 ± 12.0 mg/day azathioprine, and 8.1 ± 1.3 mg/day prednisolone.

At the start of our investigation, all groups of patients received S, the conventional formulation of CyA. Groups 1 ($n = 12$, 7 male, 5 female, mean age 43.6 ± 7.8 years) and 4 ($n = 10$, 4 male, 6 female, mean age 41.4 ± 5.3 years) did not receive any additional medication which could influence the pharmacodynamics of CyA as well as Ca^{2+} metabolism. Group 2 ($n = 10$, 5 male, 5 female, mean age 45.6 ± 8.1 years) received the CCB Phenihydine (Grindex, Latvia) at a dose of 20 mg/day (divided into two equal doses). Group 3 ($n = 10$, 6 male, 4 female, mean age 43.8 ± 7.7 years) had received a dose of 40 mg/day Phenihydine for at least 2 months prior to entering the study, and this dosage remained constant during the period of investigation. Ten volunteers were investigated in order to establish the normal values for healthy persons.

During a 2-week run – in phase the baseline was established (creatinine, urea, sodium, potassium, phosphate, alkaline phosphatase activity, parathyroid hormone level, intra- and extracellular Ca^{2+}). At the third week, patients in groups 1–3 were converted from S to SN in a 1:1 manner and then followed up for 12 weeks. Group 4 (control) continued with S.

Blood samples were taken 12 h after last taking CyA. Plasma was used for routine biochemical tests. Plasma creatinine, urea, sodium, potassium, alkaline phosphatase activity, and phosphate were determined by standard laboratory methods using an Abbot Spectrum Series II autoanalyzer. In addition, the content of Ca^{2+} in blood plasma was determined by atom-absorption spectrophotometry (model 403, Perkin Elmer).

For determination of iCa^{2+}, erythrocytes were isolated [6]. Erythrocytes were washed 4 times in 0.15 NaCl to yield 5 ml packed washed cells. Erythrocytes were hemolysed by adding a trichloroacetic acid and lanthanum chloride solution, achieving a suspension containing 50% of cells in 10% trichloroacetic acid. The supernatant was analyzed for iCa^{2+} concentration, which was determined as described above. iCa^{2} was expressed in mmol/l of packed erythrocytes. Evaluated parameters were assessed before and after 6 and 12 weeks of conversion from S to SN.

Statistical analysis was performed using a two-sample Student's t-test. All P values were two-tailed and a P value less than 0.05 was considered to indicate a significant difference. All results were expressed as mean ± SD.

Results

The iCa^{2+} level in RBC at entry was significantly ($P < 0.01$) higher in CyA-treated patients (groups 1–4) than the normal value for healthy persons (Table 1). The conversion to SN normalized the RBC iCa^{2+} level in patients from groups 1–3 4–6 weeks after the transfer and this level remained stable until the end of the study. iCa^{2+} stayed elevated in group 4 patients (Table 1).

At the same time, a transitory, statistically significant ($P < 0.05$) increase in plasma Ca^{2+} was registered in patients of group 1, the beginning of which coincided with Ca^{2+} normalization in erythrocytes.

Differences between the groups regarding creatinine, urea, intracellular parathyroid hormone levels, phosphatemia dynamics, and alkaline phosphatase activity were not discovered.

Discussion

CyA has dramatically improved the success rates for all forms of organ transplantation. However, its use is complicated by the frequent occurrence of reversible nephrotoxicity which is, to a great extent, caused by vasoconstriction and other mechanisms, largely due to elevated iCa^{2+} levels [4, 8]. The cause of elevated iCa^{2+} has not been fully elucidated. Several explanations have been proposed, including CyA – induced abnormalities in Na^{+}-Ca^{2+} exchange, decreased Ca^{2+}, Mg^{2+}-ATPase activity and suppressed ATP synthesis, increased circulating levels of calcitropic hormones (parathyroid hormone and calcitriol), and alterations in the activity or amount of the Ca^{2+}-binding protein, calmodulin [12].

At the same time, application of CCBs to patients who have undergone renal [9] and heart [3] transplantation and who have received CyA could prevent the development of nephrotoxicity symptoms. CCBs may counteract the direct vasoconstrictive effect of CyA

[10] and other vasoconstrictors, such as endothelin and tromboxan, which may be stimulated by CyA [14], to help minimize the effects of excess Ca^{2+} influx after ischemic injury of cells [13].

Our results concerning the dynamics of Ca^{2+} concentrations in erythrocytes confirm a well known phenomenon of iCa^{2+} increase under the influence of S. However, data about the normalization of this index during the switch of patients from S to SN is more interesting. This phenomenon could be a result of positive changes of CyA pharmacodynamics caused by its new microemulsion formulation, leading to restoration of some iCa^{2+} extrusion mechanisms, such as the specialized Ca^{2+} pump activity [12], which is confirmed by the transitory growth of calciemia. Evaluation in prospective controlled studies should be undertaken in order to correctly assess the mechanism of this action.

Surprisingly, we observed the same statistically significant decrease of the elevated iCa^{2+} concentration during the conversion from S to SN alone as well as during the conversion from S to SN in CCB-pretreated patients. Although the role of CCBs in the protection against chronic CyA nephrotoxicity is still under discussion, this could be an argument to focus further long-term investigations on the ability of CCBs to act as cytoprotective and nephroprotective agents during immunosuppressive protocols with the new microemulsion formulation of CyA.

References

1. Agarwal RP, McPherson RA, Treatte GA (1986) Evidence of a cyclosporine-binding protein in human erythrocytes. Transplantation 42: 627–632
2. Ahlmen J, Sundberg A, Gustavsson A, Strombom U (1995) Decreased nephrotoxicity after the use of a microemulsion formulation of cyclosporin A compared to conventional solution. Transplant Proc 27: 3432–3433
3. Carrier M, Pelletier G, White M, Bois D, Pelletier LC (1994) Effect of pentoxifylline on renal toxicity of cyclosporine after heart transplantation. Preliminary results of a clinical trial. The Third International Congress on Cyclosporine, Abstract 340
4. Cristians U, Sewing KF (1993) Cyclosporin metabolism in transplant patients. Pharmacol Ther 57: 291–345
5. Fujita T (1986) Aging and calcium. Miner Electrolyte Metab 12: 149–156
6. Gafter U, Malachi T, Barak H, Djaldetti M, Levi J (1989) Red blood cell calcium homeostasis in patients with end-stage renal disease. J Lab Clin Med 114: 222–231
7. Gonzalez JM, Sui WN (1995) Cell calcium and arterial blood pressure. Semin Nephrol 15: 564–588
8. Hawkins RG, Burgess ED (1992) Hypertension in transplant recipients. In: Leendert CP, Solez K (eds) Organ transplantation: long-term results. Marcel Dekker, New York, pp 303–318
9. Hull AR (1994) Present dilemmas in the clinical use of cyclosporin. In: Neoral: a new formulation of cyclosporin. World Medical Press, New York, pp 6–11
10. Huntress JD, Papadakos PJ (1996) The role of calcium-channel antagonists in solid organ transplantation. New Horiz 4: 129–133
11. Kahan BD (1994) Role of cyclosporine: present and future. Transplant Proc 26: 3082–3087
12. Salducci MD, Chauvet-Monges AM, Dussol BM, Berland YF, Crevat AD (1995) Is the beneficial effect of calcium channel blockers against cyclosporine A toxicity related to a restoration of ATP synthesis? Pharmacol Res 12: 518–522
13. Schrier RW (1991) Role of calcium channel blockers in protection against experimental renal injury. Am J Med (Suppl 5a) 90: 21–26
14. Weir MR (1991) Therapeutic benefits of calcium channel blockers in cyclosporine treated organ transplant recipients. Blood pressure control and immunosuppression. Am J Med (Suppl 5a) 90: 32–36