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In Vitro Effects of Cyclosporine and FK 506 on the Renal Cortex

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CYCLOSPORINE (CyA) and FK 506 are the effective immunosuppressive agents used in human organ transplantation and a variety of autoimmune diseases.[1-3] The nephrotoxicity of CyA is well known.[4,5] Preliminary reports indicate the nephrotoxic nature of FK 506, though the clinical significance is yet to be established, especially in view of the favorable side effect profile that FK 506 possesses.[6-8] The mechanism of nephrotoxicity of CyA is not clearly established. Direct toxicity on the cell and cell membranes, altered prostaglandin synthesis, changes in the handling of calcium by the cells, and hemodynamic alterations have all been implicated.[9] Very little is known about the toxicity of FK 506. There is paucity of data on the direct effects of CyA and FK 506 on renal tissue. Here we report the in vitro effects of both CyA and FK 506 on the calcium sequestration by the microsomes and mitochondria of renal cortical tissue.

MATERIALS AND METHODS

Adult male, white New Zealand rabbits were killed. The kidneys were perfused in situ with HEPES (10 mmol/L) and sucrose (0.25 mol/L) medium (pH 7.35). The kidneys were removed and the cortices were dissected and homogenized in the same medium (10 mL/g). Mitochondria and microsome were isolated by high speed and ultracentrifugation as described,[10] and stored under liquid nitrogen until they were used for the studies.

The protein concentration was measured with the Biorad reagent.[11] The ATPase activity was calculated from the amount of the inorganic phosphate released in the presence of the microsomes (50 μg protein), ATP (3 mmol/L), and EGTA (1 mmol/L) with or without calcium chloride (2 mmol/L). Inorganic phosphate was measured according to the method of Fiske and Subba Row,[12] with 10% ascorbic acid as the reducing agent.

Calcium in the suspending medium was measured with the metallochrome indicator Arsenazo III.[13] The calcium concentration was continuously followed at a wavelength pair of 665 nm and 685 nm in the dual-wavelength spectrophotometer (Biomedical instrumentation, University of Pennsylvania, Johnson Research Foundation, Philadelphia, Penn). The effect of 1 to 10 μg/mL of either CyA or FK 506 on calcium sequestration was compared to the control values obtained in the presence of DMSO (10 μL). The microsomal preparation was unaffected by 1 mmol/L NaN_3. The mitochondrial calcium uptake was completely blocked by both NaN_3 (1 mmol/L) and FCCP (5 μmol/L), thus confirming the nature of the microsomal and mitochondrial preparation. The tracings from single preparation, done on the same day, are presented. At the end of 10 or 20 minutes of calcium uptake, A23187 (2 μg/mL) was added to release the sequested calcium.

RESULTS

There was a dose-dependent decrease in calcium uptake by the microsome in the presence of CyA (Fig 1A and B).

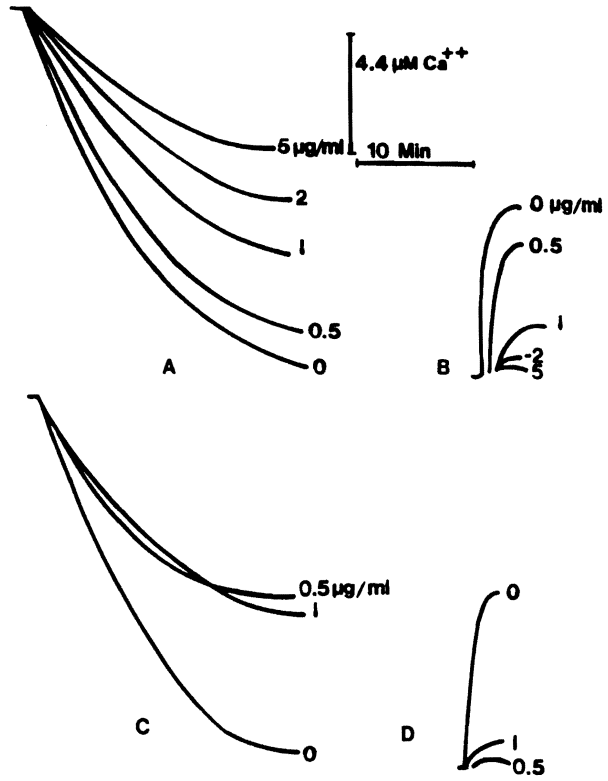

Fig 1. Effect of CyA (**A** and **B**) and FK 506 (**C** and **D**) on the microsomal calcium uptake (A and C) and A23187-induced release of calcium (B and D). Concentration of the drug is given beside each curve.

Similarly, there was a dose-dependent decrease in calcium release from microsomes following the addition of A23187 (Fig 1B). A significant decrease in calcium uptake and release by the ionophore from the microsomes was seen in the presence of FK 506 (Fig 2C and D). Higher concentrations of FK 506 (10 μg/mL) further decreased calcium uptake.

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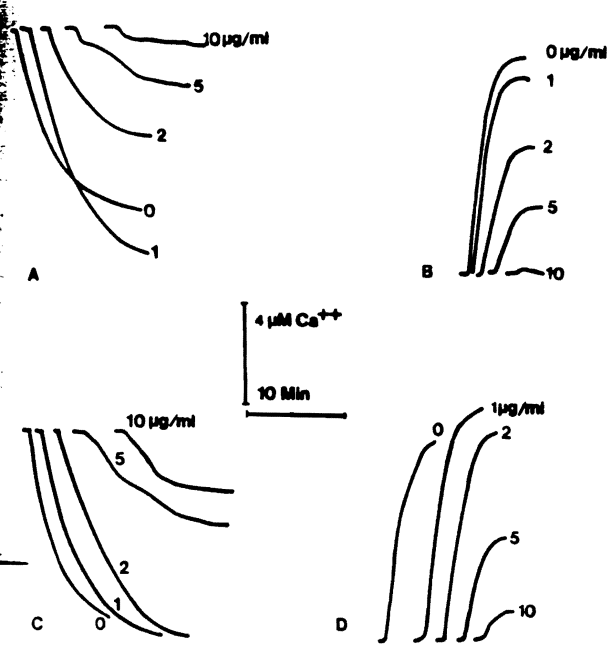

Fig 2. Effect of CyA (**A** and **B**) and FK 506 (**C** and **D**) on mitochondrial calcium uptake (A and C) and A23187-induced release (B and D). The numbers along each curve represent the concentration (µg/mL) of CyA (A and B) and FK 506 (C and D).

In the mitochondria, similar to what occurred in the microsome, there was a dose-dependent decrease in the calcium sequestration in the presence of CyA (Fig 2A and B) and FK 506 (Fig 2C and D). Furthermore, both CyA and FK 506 caused a dose-dependent decrease in A23187 releasable calcium. The effect of FK 506 was seen only at very high concentrations of the drug.

There was two- to three-fold stimulation of microsomal ATPase activity in the presence of calcium. This is suggestive of the specific nature of the enzyme studied. At the concentrations of the drugs used (1 to 10 µg/mL), neither CyA nor FK 506 had an effect on calcium-stimulated ATPase activity.

In the experiments in which mitochondria and microsomes were coincubated with the drug, $NADPH^+$ and microsomes provided the drug metabolizing activity. CyA inhibited the calcium uptake. $NADPH^+$ (0.1 mmol/L) itself had no effect on the calcium uptake, but reversed the effect of 5 µg of CyA. DTT 1 mmol/L added 10 minutes into the incubation in the presence of CyA restored the calcium-sequestrating ability. Both GSH (1 mmol/L) and DTT (1 mmol/L) were effective in preventing the inhibitory effect of the CyA. FK 506, at a concentration of 5.0 µg, had only minimal effect on calcium sequestration. Addition of $NADPH^+$ in the presence of FK 506 (0.1 mmol/L) inhibited calcium sequestration significantly. This was prevented by GSH and DTT (1 mmol/L).

DISCUSSION

The results indicate that CyA, at concentrations that are clinically encountered, is inhibitory to calcium sequestration by both the microsome and the mitochondria, isolated from the renal cortex. FK 506 also inhibited the calcium sequestration by these organelles, however, at concentrations that are much higher than the therapeutic levels reported.[7,8] It is also evident that mitochondria are relatively resistant to the effects of the CyA and FK 506, thus, concentrations of CyA and FK 506 that are inhibitory to only the endoplasmic reticulum, sparing the mitochondria, would be expected to alter the distribution of the intracellular stores of the calcium. This is consistent with the findings of Zidek and Neuman,[14] in permeabilized human neutrophils incubated in the presence of the CyA. In isolated rat hepatocytes, CyA (10 µg/mL) was shown to increase calcium flux across the plasma membrane and increase the cellular calcium content.[15] Another important finding is the effect of metabolic transformation of these drugs on their ability to inhibit the calcium sequestration by the cellular organelles. The data clearly indicate conversion of CyA to an inactive metabolite (S) and FK 506 to an active metabolite in the presence of the microsomal drug-metabolizing system. Further, a critical role for sulfhydryl groups is suggested by the protective effects of the sulfhydryl reagents on CyA and the FK 506 inhibition of the calcium sequestration by the cellular organelles.

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