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Receptor-mediated Cellular Uptake of Cyclosporine

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THE PROCESS of cellular uptake and metabolism of cyclosporine (CsA) is poorly understood. Earlier studies in this laboratory indicated that transport of CsA in blood occurs in association with plasma lipoproteins, with low density lipoprotein (LDL) and high density lipoprotein (HDL) carrying about 32% each of the total CsA in blood. This observation suggests the possibility that the cellular uptake of CsA may occur through the LDL receptor pathway. To further identify this phenomenon, we incubated CsA-LDL complex with freshly isolated peripheral blood lymphocytes (PBL) and also with PBL in which LDL receptors synthesis was enhanced by incubation in lipid free serum. Results of these experiments show that cellular uptake of CsA is increased about 25% in cells incubated in lipid-free serum relative to controls. This increased uptake of CsA could be in part due to the proliferation of LDL receptors in the stimulated cells. Since at least 32% of the drug circulates in association with LDL, this pathway provides an important mechanism for the metabolism of CsA.

The aims of this study were to examine the kinetics of binding and uptake of CsA by the human peripheral blood lymphocytes, and the enhancement of CsA uptake by peripheral blood lymphocytes via LDL receptor pathway.

Cyclosporine (CsA) is widely used in transplantation as the immunosuppressive agent of choice. In our earlier studies, we have shown that transport of CsA in blood occurs mainly in association with plasma lipoproteins, 70% of the drug being in LDL and HDL fractions.[1] This suggests that cellular uptake and metabolism of CsA may, at least in part, follow the lipoprotein metabolism, specifically, the LDL-receptor mediated pathway.

MATERIALS AND METHODS

Tritiated dihydrocyclosporine A (CsA) (60 mCi/mmol) and CsA standards were obtained from Sandoz, Ltd (Basel, Switzerland) as ethanolic solutions. FK506 was a gift from Fujisawa Pharmaceutical Co, Ltd (Osaka, Japan). Methanolic solution of FK506 was used in the experiments. Culture media and antibiotics were obtained from GIBCO (Grand Island, NY). All other reagents and supplies were from Fisher Scientific Co, Pittsburgh.

Human LDL (d = 1.019 to 1.063) was prepared from blood collected in 0.1% EDTA from healthy subjects who had fasted overnight. Lipoproteins were fractionated by sequential floatation in a Beckman preparative ultracentrifuge (Beckman Instruments, Inc, Fullerton, CA) according to Havel et al.[2] Protein determinations were performed by the Bradford method.

Peripheral blood mononuclear cells were isolated as previously described[3] from approximately 100 ml of blood obtained from healthy volunteers for each experiment. The monocyte content of these cells was depleted by the method of Jerrells et al. Cell viability was assessed by using the dye exclusion method.

LDL (100 μg in 1 ml RPMI 1640 medium) was added dropwise with stirring to an ethanolic [^{3}H] CsA solution (50 μl). The mixture was incubated with constant shaking for one hour at room temperature. The specific activity of the [^{3}H] CsA-LDL complex thus obtained in three separate preparations was 20 ± 0.8 × 10^4 dpm/μg LDL.

Lymphocytes were incubated in RPMI 1640 medium containing 1% bovine serum albumin (BSA) in the presence or absence of CsA and/or FK506 for 24 hours. After these preincubations, the cells were washed twice with ice-cold 0.85% NaCl solution and then resuspended in 1% BSA-RPMI medium. The cells were placed in culture wells (10^6 cells/well) and exposed to increasing molar concentrations of [^{3}H] CsA (specific activity, 900 to 1,100 dpm/pmol) and equivalent concentrations of cold CsA and FK506, as indicated in the legends to the figures, for one hour at 37°C in the humidified atmosphere of 95% O_2:5% CO_2. Transport flux was stopped by adding ice-cold saline and cooling the mixture further for ten minutes over ice. The cells were washed three times with cold saline and incubated in 0.2 mL 1 N KOH for one hour at 70° C. The digested solution was used for the measurement of radioactivity and protein determinations.

RESULTS

CsA uptake by lymphocytes from two healthy donors was studied by incubating cells with the [^{3}H] CsA-LDL complex. The kinetics of the uptake of CsA in these experiments are shown in Figure 1, panels A and B. In both individuals studied, the CsA uptake appears to reach saturation at an LDL concentration of about 50 μg/ml medium. The corresponding CsA concentration is about 900 ng/mL. In a previous study[3] on the cell surface binding and subsequent metabolism of LDL by T and B lymphocytes, we have observed that the LDL receptor is saturated at 50 μg LDL/ml medium. Moreover, [^{3}H] CsA uptake by lymphocytes was suppressed in the presence of cold CsA and LDL. Combined, these observations suggest that internalization of lipoprotein-associated CsA may occur via the high-affinity specific receptor for LDL on the cell surface. The experimental conditions used in these studies imply that the CsA measurements shown reflect both the processes of cell surface binding and internalization.

When the cell content of CsA was compared in an experiment in which two sets of lymphocytes obtained from the same donor were incubated simultaneously with various concentrations of CsA alone or with CsA-LDL complex it was observed that cells incubated with CsA-LDL contained greater amounts of CsA at each of the concentrations studied. Quite importantly, internalization of CsA from CsA-LDL complex during the initial phase was 45% higher

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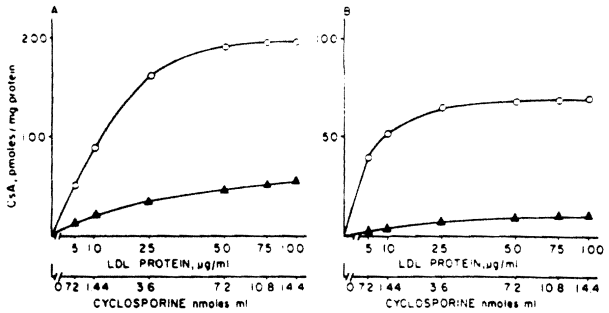

Fig. 1. Cellular uptake of CsA in PBL incubated with the [^{3}H] CsA-LDL complex for one hour. The open and closed symbols represent the total and nonspecific (LDL receptor-independent) uptake of [^{3}H] CsA, respectively. Panels A and B are results in two subjects.

relative to control. When the receptor and internalization processes were saturated this difference stabilized at 18% as shown in Figure 2.

Cellular CsA uptake in the absence of LDL was studied in lymphocytes from two healthy volunteers (other than for Figs 1 and 2). The results of the experiments shown in Fig 3, panels A and B, indicate that, as in the presence of LDL, [^{3}H]CsA uptake in this instance may also involve a saturable process.

DISCUSSION

The cellular uptake of CsA appears to involve both specific was well as nonspecific processes, the former taking place when the drug is associated with lipoprotein. In this instance, the CsA-LDL complex may be internalized via the high-affinity, specific cell surface receptor for LDL.

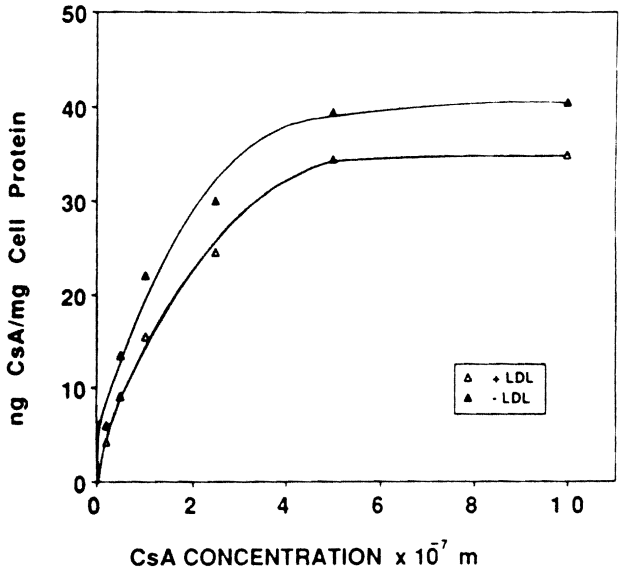

Fig. 2. Lymphocytes were preincubated in 1% BSA in RPMI 1640 for 24 hours. Cells were then exposed with CsA (△—△) and CsA • LDL complex (▲—▲) and cellular uptake of [^{3}H] CsA was measured.

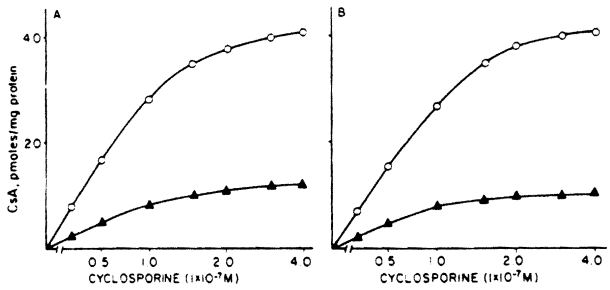

Fig. 3. Cellular uptake of CsA in PBL incubated with [^{3}H] CsA (○—○) and unlabeled CsA (▲—▲). Panels A and B are results in two subjects.

Support for this concept also derives partly from the calculations of dissociation constants for various experiments reported here. The double reciprocal plots of data from these experiments yield two quite distinct apparent dissociation constants (K_D). For the cellular uptake of CsA complexed with LDL, K_D = 2.3 and 0.6 x 10^{-9} mol/l (Fig 1, panels A and B, respectively). For the data in all of the rest of the experiments, K_D = 2 x 10^{-7}mol/l. The K_D of 10^{-9} mol/l for CsA when in association with LDL certainly suggests high-affinity binding. The rate-limiting step in the LDL pathway is the binding of LDL to a specific high-affinity receptor on the cell surface.[4] It may be expected, therefore, that this would be the initial step in the metabolism of CsA bound to LDL. The significantly reduced cellular content of [^{3}H] CsA in the presence of cold LDL appears to strengthen this view. Whether such a binding of CsA-LDL to the LDL receptor followed by further metabolism along the LDL pathway occurs cannot be inferred from the experiments reported here. If true, however, it could represent a significant pathway for CsA metabolism because some 32% of the drug in blood is bound to plasma LDL.[1] The concentrations of CsA required to saturate the cellular mechanisms(s) responsible for CsA uptake are also different in the presence and absence of LDL, 900 ng/ml with LDL and about 300 ng/ml without LDL. This information further segregates the two processes.

CONCLUSIONS

Cyclosporine uptake by peripheral blood lymphocytes may involve specific as well as nonspecific processes. In either case, the kinetics of uptake indicate a saturable phenomenon implying the involvement of a "carrier" or "transport" protein. Cyclosporine uptake by cells when associated with LDL was greater relative to the uptake of cyclosporine alone. The dissociation constant for CsA-LDL complex of 10^{-9} M suggests high affinity binding, perhaps to the LDL receptor.

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