Isolation, identification and immunosuppressive activity of SDZ-IMM-125 metabolites from human liver microsomes

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SUMMARY

SDZ-IMM-125 N-methyl leucine 9 hydroxylated in the γ position is a metabolite which was extracted from incubated human liver microsomes and subsequently separated by normal and reverse-phase HPLC. This metabolite was identified by fast atom bombardment mass spectrometry, electrospray-ms/ms mass spectrometry and nuclear magnetic resonance spectroscopy. The in vitro 50% inhibitory concentration, tested against bidirectional mixed lymphocyte reaction was 80 μg/l indicating that this metabolite does not retain in vitro immunosuppressive activity most probably due to the structural modification of SDZ-IMM-125 in the recognized binding region to cyclophilin A reducing its binding affinity relative to the parent drug.

INTRODUCTION

Cyclosporin A (CyA), has been regarded as the prototype of a new generation of immunosuppressive agents (1) but its adverse effects on the kidney, the liver and the arterial blood pressure (2), limit its more widespread clinical use. Consequently, new analogues or other drugs of different structures (3) were developed with the goal of overcoming the adverse side effects in the clinical use of CyA.

SDZ-IMM-125 is a new cyclosporin resulting from the hydroxyethyl modification of D-serine-8 cyclosporin that is obtained by fermentation of Tolypocladium inflatum in medium enriched in D-serine. This modification does not alter the interaction of the cyclophilin complex with
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II (5 g/l) were purchased from Boehringer Mannheim (Mannheim, Germany). The matrix used in FAB/MS, 3-nitrobenzyl alcohol, was manufactured by Aldrich-Chemie (Steinheim, Germany). Demineralized and filtered (Milli-Q water purification system; Millipore-Waters, Milford, MA, USA) water was used. All cell culture reagents were obtained from Gibco Laboratories (Paisley, UK).

Human liver

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Preparation of human liver microsomes

A piece of liver was weighted, washed with ice-cold 3 mmol/l imidazole homogenizing buffer containing 0.5 mol/l sucrose, blotted with filter paper, and minced with scissors. The minced liver was treated and fractionated according to the method of Amar-Costesc et al. (8). Protein and cytochrome P-450 concentrations were determined according to standard published procedures (9,10).

IMM-125 microsomal incubation and extraction of metabolites

The NADPH-generating medium (2 ml) containing 1.76 mg of NADPH, 5.07 mg of NADP+, 0.4 ml of MgCl2 (0.5 mol/l), 30 mg of glucose 6-phosphate and 1.6 ml of Tris buffer (pH 7.4) was pre-incubated in a Gallenkamp (Grant Instruments, Cambridge, UK) shaking incubator for 15 min at 37°C in small Erlenmeyer flasks. To this solution we added 2.5 ml of human liver microsomes (8.3 mg protein/ml), 12 μl (5 g/l) glucose-6-phosphate dehydrogenase (specific activity 350 kU/g), and 62.5 μg IMM-125 dissolved in acetonitrile (25 μl). This mixture was incubated for 4 h at 37°C. After the elapsed time and transfer of the incubation medium to a centrifuge tube, 7 ml of dichloromethane was added. After 2 min of vortex-mixing, the sample was centrifuged for 10 min at 1150 g. The aqueous phase was discarded and the residue remaining after evaporation of the dichloromethane phase was dissolved in 1.5 ml acetonitrile:water (3:7, v/v). The resulting solution was then washed with 1.5 ml hexane (2 min of vortex mixing); the hexane layer was discarded after centrifugation for 5 min at 1150 g. The acetonitrile:water phase was extracted again with 3 ml of dichloromethane. After 2 min of vortex-mixing and centrifugation for 10 min at 1150 g the water phase was