In vitro interaction between cyclosporin A and macrolide antibiotics

It is now well established, both *in vitro* and *in vivo*, that certain macrolide antibiotics inhibit cyclosporin A metabolism, which is catalyzed by a cytochrome P-450 belonging to the P4503A subfamily (Combalbert *et al.*, 1989). Clinical studies have shown that pharmacokinetic interactions occur between cyclosporin A and erythromycin (Kohan, 1986), josamycin (Kreft-Jais *et al.*, 1987), and roxithromycin (Billaud *et al.*, 1990), although spiramycin does not affect cyclosporin A pharmacokinetics (Vernillet *et al.*, 1989). *In vitro*, erythromycin, troleandomycin and josamycin inhibit cyclosporin A oxidation (Pichard *et al.*, 1990), whereas roxithromycin does not seem to interfere with the microsomal cytochrome P-450 system (Delaforge *et al.*, 1988). We have evaluated the potential inhibitory effect of rokitamycin, a new 16-membered macrolide antibiotic, on human liver microsomal cyclosporin A metabolism. The effects of erythromycin, josamycin, roxithromycin, spiramycin and troleandomycin were also studied for comparative purposes.

Microsomes were prepared from a single human liver as described previously (Marre *et al.*, 1992). This liver was previously characterized for its CYP3A activity (Lacarelle *et al.*, 1991). The use of the sample was authorized by the French National Ethics Committee when the liver could not be used for transplantation. The inhibition of cyclosporin A oxidation by the six macrolide antibiotics was measured according to Pichard *et al.* (1990). Microsomes (1 mg protein ml^{-1}) were incubated with 1 μM cyclosporin A, NADPH (1 mM), and 100 μM of each macrolide for 30 min at 37° C. Full kinetic studies were also performed to determine apparent K_i values. Thus, microsomes were incubated for 20 min with 1, 2.5 and 5 μM cyclosporin A and five different concentrations of each macrolide (2.5, 5, 10, 50, 100 μM for erythromycin, josamycin, rokitamycin, roxithromycin and troleandomycin; 250, 500, 1000, 5000, 10 000 μM for spiramycin). After addition of methanol, samples were centrifuged (10 000 *g*, 2 min). Supernatants were analysed by h.p.l.c. according to Fabre *et al.* (1987). Since production of the main metabolites (M1, M17, M21) appeared to be mediated by the CYP3A family (Combalbert *et al.*, 1989), we expressed cyclosporin A oxidase activity as the amount of these metabolites produced per time unit and mg of microsomal protein. Apparent K_i values were obtained from linear regression analysis of Dixon plots. All drugs were dissolved in solutions containing < 1% w/v dimethyl sulphoxide, at which concentration no inhibition of metabolism due to the solvent was observed.

All of the macrolides (100 μM), except spiramycin, inhibited cyclosporin A oxidation, by 33% for erythromycin, 80% for josamycin, 59% for rokitamycin, 74% for roxithromycin and 83% for troleandomycin (Table 1). Apparent K_i values obtained from Dixon plots were as follows: erythromycin, 57 μM, josamycin, 12 μM, rokitamycin, 30 μM, roxithromycin, 113 μM, spiramycin, 6300 μM and troleandomycin, 17 μM.

Table 1 Results of screening macrolide antibiotics as inhibitors of cyclosporin A oxidase activity using human liver microsomes

	*CsA oxidase activity** (%)	K_i (μM)
Control	100	
Macrolides:		
Erythromycin	67	57
Josamycin	20	12
Rokitamycin	41	30
Roxithromycin	26	113
Spiramycin	100	6300
Troleandomycin	17	17

*Results, expressed as % of uninhibited activity, are the mean of three determinations of a single liver. Macrolide concentration = 100 μM.

Thus, rokitamycin, like erythromycin, josamycin, roxithromycin and troleandomycin, inhibited *in vitro* cyclosporin A metabolism, while spiramycin had no effect. These *in vitro* findings were in agreement with those of clinical studies. (Billaud *et al.*, 1990; Kohan, 1986; Kreft-Jais *et al.*, 1987). K_i values obtained for josamycin, erythromycin and troleandomycin were close to those reported by other authors (Pichard *et al.*, 1990). The K_i value of rokitamycin, 30 μM, was lower than those of erythromycin and roxithromycin and higher than those of josamycin and troleandomycin. This suggests that, *in vivo*, rokitamycin could be a more potent inhibitor than erythromycin or roxithromycin. However, as shown by Dixon plots, the inhibitory effect of erythromycin and rokitamycin was detectable when the cyclosporin A/erythromycin concentration ratio was between 1/10 and 1/50 and when the cyclosporin A/ rokitamycin concentration ratio was between 1/20 and 1/50, whereas *in vivo* tough blood erythromycin concentrations are around 1 μM (Fourtillan *et al.*, 1983) and those of cyclosporin A between 0.1 and 0.25 μM (Ptachcinski *et al.*, 1985). The presumed blood drug concentration ratio is, therefore, estimated to be 1/4 to 1/10 for erythromycin. Precise prediction of *in vivo* interactions from *in vitro* dates requires estimates of the intracellular cyclosporin/macrolide concentration ratio. Therefore, our *in vitro* results must be extrapolated with caution regarding extrapolation to an *in vivo* effect of rokitamycin on cyclosporin A metabolism.

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References

Billaud, E. M., Guillemain, R., Fortineau, N., Kitzis, M. D., Dreyfus, G., Amrein, C., Kreft-Jais, C., Husson, J. M. & Chretien, P. (1990). Interaction between roxithromycin and cyclosporin in heart transplant patients. *Clin. Pharmacokin.*, **19**, 499–502.

Combalbert, J., Fabre, I., Fabre, G., Dalet, I., Derancourt, J., Cano, J. P. & Maurel, P. (1989). Metabolism of cyclosporin A. IV. Purification and identification of the ripampicin-inducible human liver cytochrome P-450 (cyclosporin A oxidase) as a product of P450IIIA gene subfamily. *Drug Metab. Dispos.*, **17**, 197–207.

Delaforge, M., Sartori, E. & Mansuy, D. (1988). *In vivo* and *in vitro* effects of a new macrolide antibiotic roxithromycin on rat liver cytochrome P-450: Comparison with troleandomycin and erythromycin. *Chem. Biol. Interact.*, **68**, 179–188.

Fabre, G., Bertault-Peres, P., Fabre, I., Maurel, P., Just, S. & Cano, J. P. (1987). Metabolism of cyclosporin A. I Study in freshly isolated rabbit hepatocytes. *Drug Metab. Dispos.*, **15**, 384–390.

Fourtillan, J. B., Bryskier, A. and Lefebvre, M. A. (1983). Etude pharmacocinetique de l'interaction tetracycline-propionate d'erythromycine. *Med. Mal. Infect.*, **13**, 809–813.

Kreft-Jais, C., Billaud, E. M., Gaudry, C. & Bedrossian, J. (1987). Effect of josamycin on plasma cyclosporin levels. *Eur. J. clin. Pharmac.*, **32**, 327–328.

Kohan, D. E. (1986). Possible interaction between cyclosporin and erythromycin. *New Engl. J. Med.*, **314**, 448.

Lacarelle, B., Marre, F., Blanc-Gauthier, T., Zhou, X. J., Placidi, M., Catalin, J. & Rahmani, R. (1991). Use of human and animal liver microsomes in drug metabolic studies. *Eur J. Drug Metab. Pharmacokin.*, Special Issue III, 458–465.

Marre, F., Fabre, G., Lacarelle, B., Bourrie, M., Catalin, J., Berger, Y., Rahmani, R. & Cano, J. P. (1992). Involvement of the cytochrome P-450IID subfamily in minaprine 4-hydroxylation by human hepatic microsomes. *Drug Metab. Dispos.*, **20**, 316–321.

Pichard, L., Fabre, I., Fabre, G., Domergue, J., Saint Aubert, B., Mourad, G. & Maurel, P. (1990). Cyclosporin A drug interactions. Screening for inducers and inhibitors of cytochrome P-450 (Cyclosporin A oxidase) in primary cultures of human hepatocytes and in liver microsomes. *Drug Metab. Dispos.*, **18**, 595–606.

Ptachcinski, R. J., Carpenter, B. J., Burckart, G. J., Venkataramanan, R. & Rosenthal, J. T. (1985). Effects of erythromycin on cyclosporin levels. *New Engl. J. Med.*, **313**, 1416–1417.

Vernillet, L., Bertault-Peres, P., Berland, Y., Barradas, J., Durand, A. & Olmer, M. (1989). Lack of effect of spiramycin on cyclosporin pharmacokinetics. *Br. J. clin. Pharmac.*, **27**, 789–794.