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Comparison of the In Vitro Metabolism of the Macrolide Immunosuppressants Sirolimus and RAD

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RAD [40-*O*-(2-hydroxyethyl)-rapamycin] is a novel macrolide immunosuppressant that is structurally related to sirolimus.[1] Because of its immunosuppressive synergistic interaction,[2] RAD is under clinical investigation as an immunosuppressant, in combination with cyclosporine, for posttransplantation therapy. It was our goal to study the oxidative metabolism of sirolimus and RAD, to identify the structures of their metabolites, and to evaluate the impact of the 40-*O*-2-hydroxyethyl group on the in vitro metabolism of RAD in comparison with sirolimus.

METHODS

Sirolimus or RAD was incubated with human liver microsomes. The metabolites were separated and isolated using HPLC and their structures elucidated using MS/MS in combination with collision-induced dissociation and ion-trap MS (MS^n). The cytochrome P450 (CYP) enzymes involved were identified using isolated cDNA-expressed human CYP enzymes. Sirolimus, RAD, and their metabolites were quantified using LC/LC-MS.

RESULTS AND DISCUSSION

A total of 12 sirolimus and 13 RAD metabolites were isolated and the structures of 10 sirolimus (46-, 24-, 25-, 12-, 11-, 14-, and 49-hydroxy; 39-*O*-, 27-*O*-, and 16-*O*-desmethyl sirolimus) and 11 RAD metabolites (as for sirolimus + 40-*O*-deshydroxyethyl RAD) were proposed based on the analysis of MS fragmentation patterns. CYP3-A4, -3A5, and -2C8 were the major CYP enzymes involved in sirolimus and RAD metabolism (Fig 1). Based on the metabolism sites, five groups of metabolites were differentiated. CYP enzymes showed different preferences for these metabolism sites (Fig 1). The RAD 40-*O*-2-hydroxyethyl group not only inhibited 39-*O*-demethylation, the major metabolism reaction of sirolimus, but also decreased hydroxylation in the C10 to C14 region, which is the major hydroxylation region of sirolimus (Fig 1). In addition to the metabolic pathways similar to sirolimus, RAD was dehydroxyethylated by CYP-3A, resulting in rapamycin. Based on intrinsic clearance, it was estimated that dehydroxyethylation is only a minor, most likely clinically insignificant, RAD metabolic pathway. In vitro, 5.1% was converted to rapamycin. However, if extrapolated to the in vivo situation, this is most likely an overestimation, because our in vitro assay ignored that: (a) there are probably more RAD metabolites generated than have been isolated in our study; (b) there is most likely significant metabolism of rapamycin to its inactive metabolites in vivo; and (c) there may be significant degradation of both RAD and rapamycin in vivo. The total intrinsic formation clearance of RAD metabolites by human liver microsomes was 2.7-fold lower than that of sirolimus (mean 682 vs 1810 mL/g·min). We conclude that, although structurally similar, in vitro metabolism of RAD and sirolimus shows significant differences.

REFERENCES

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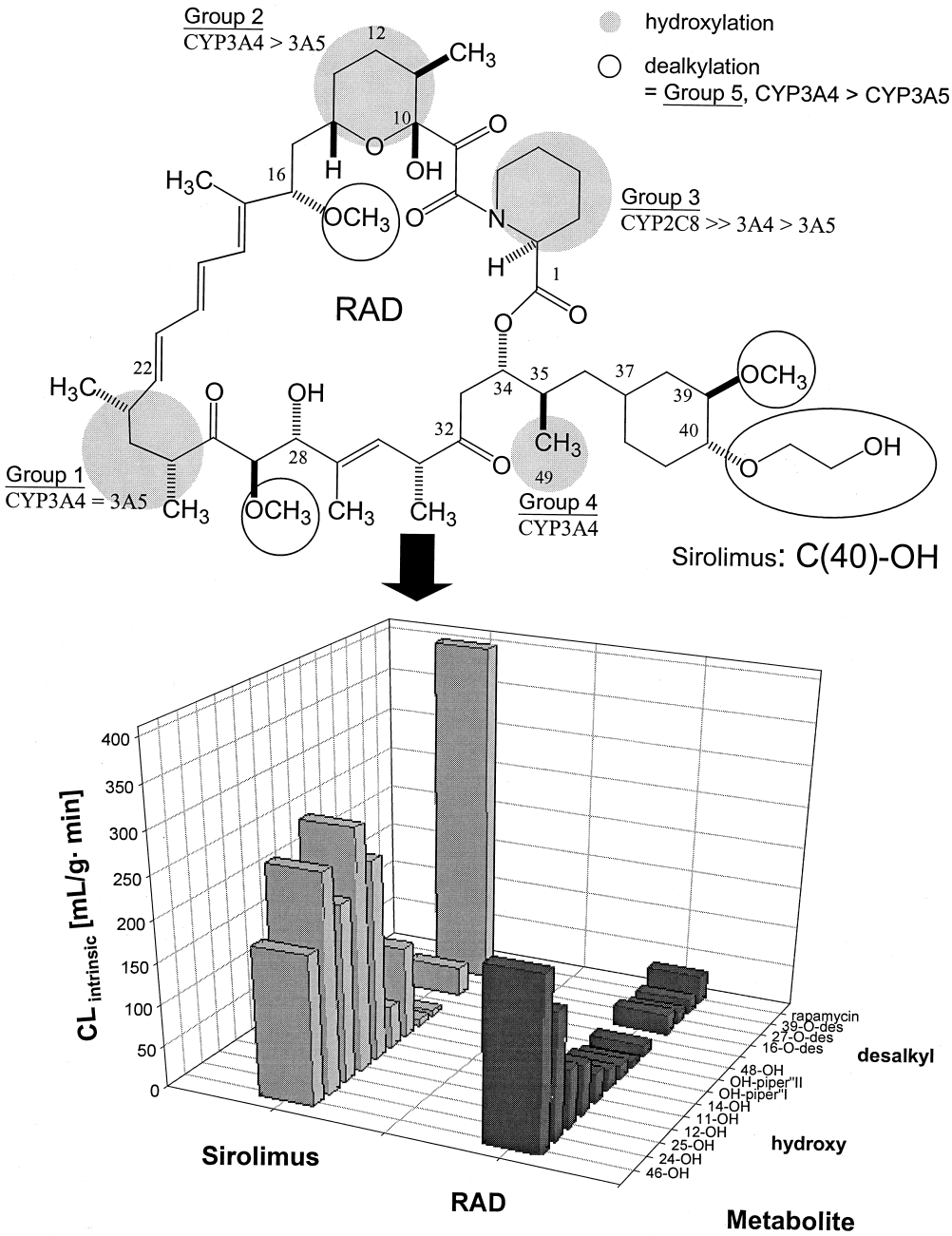

Fig 1. Comparison of in vitro metabolism of sirolimus and RAD by human liver microsomes. **(Top)** Major drug metabolism sites and cytochrome P450 enzymes. **(Bottom)** Comparison of intrinsic formation clearances of individual sirolimus and RAD metabolites. Numbering of the molecules follows IUPAC guidelines.