Response to Zhou et al.

Osteomalacia is a frequent complication resulting from long-term therapy with drugs such as phenytoin, carbamazepine, and phenobarbital. We have investigated the crosstalk between pregnane X receptor (PXR) and vitamin D receptor (VDR) and have reported that (a) PXR binds to and transactivates vitamin D-responsive elements in the 25-hydroxyvitamin D₃-24-hydroxylase (CYP24) promoter, and (b) PXR agonists increase CYP24 mRNA expression in human hepatocytes and mouse kidney (1). Since the CYP24 enzyme converts 1,25-dihydroxyvitamin D₃ to inactive metabolites, these observations provide an objective explanation for the induction of osteomalacia by PXR agonists. In their report on this crosstalk in the June issue of the *JCI*, Zhou et al. (2) concluded instead that CYP24 is not induced but rather downregulated by PXR agonists. These authors observed neither binding to nor transactivation of the CYP24 promoter by PXR, nor did they find induction of CYP24 mRNA in human hepatocytes. As

soon as we became aware of this work, we repeated key experiments using extracts from new hepatocyte cultures and new inducers (SR128123 and CITCO). Moreover, we evaluated the constitutive androstane receptor-VDR (CAR-VDR) crosstalk. Our new results fully confirm our previous findings. In human hepatocyte cultures, the CYP24 mRNA level was increased by rifampicin and 2 other PXR agonists, phenobarbital and SR128123. EMSA not only confirmed the binding of PXR to vitamin D-responsive elements-I and -II of CYP24 but also revealed that CAR binds these responsive elements. This last point is consistent with the finding that CITCO, a specific agonist of CAR, was a weak inducer of CYP24 in hepatocytes and that androstanol, an inverse agonist of CAR, inhibited CAR-mediated transactivation of the CYP24 promoter. We believe that these discrepancies between our work and that published by Zhou et al. (2) could be due to methodological differences in hepatocyte cultures (culture medium), transfection assays (cell lines), and EMSA. More work

is currently being done to identify the origin of these discrepancies.

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