Oxidation of Midazolam and Triazolam by Human Liver Cytochrome P450IIIA4.

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SUMMARY

The metabolism of midazolam and triazolam to their 1′-hydroxy and 4-hydroxy metabolites was studied in microsomes of 15 human livers. The formation of both metabolites was inhibited by more than 90% by an antiserum directed against a pregnenolone 16α-carbonitrile-inducible cytochrome P450 (P450PCN1) of rat liver. Moreover, midazolam hydroxylase activity was immunoprecipitated from solubilized human microsomes with polyclonal antibodies against rat P450PCN1 and the closely related human isozyme P450NF. A close correlation was observed between the amount of protein detected in immunoblots with these antibodies and the midazolam or triazolam hydroxylase activity. The formation of both metabolites of midazolam was inhibited by triacetyloleandomycin, a known inhibitor of cytochromes P450 of the IIIA family. Direct evidence that P450IIIA4 catalyzes the metabolism of midazolam was provided through the use of cDNA-directed expression. Monkey COS cells transfected with human P450PCN1 cDNA were able to catalyze both the 1′- and the 4-hydroxylation of midazolam. We conclude that the metabolism of midazolam and triazolam in human liver is predominantly mediated by cytochrome P450IIIA4. Two of 15 human livers expressed a second immunoreactive microsomal protein of higher apparent *M*, and were more active in midazolam 1′-hydroxylation. Our data also provide evidence that the marked interindividual variation in the response to these widely used benzodiazepine drugs is due to variable hepatic metabolism.