Fakultät für Medizin

Dokumenttyp: journal article

Autor(en) des Beitrags:
Yang, TJ; Krausz, KW; Shou, M; Yang, SK; Buters, JT; Gonzalez, FJ; Gelboin, HV

Titel des Beitrags:
Inhibitory monoclonal antibody to human cytochrome P450 2B6.

Abstract:
The human cytochrome P450 2B6 metabolizes, among numerous other substrates, diazepam, 7-ethoxycoumarin, testosterone, and phenanthrene. A recombinant baculovirus containing the human 2B6 cDNA was constructed and used to express 2B6 in Sf9 insect cells. The 2B6 was present at 1.8 +/- 0.4% of the total cellular protein and was purified to a specific content of 13.3 nmol/mg protein. Mice were immunized with the purified 2B6, and a total of 811 hybridomas were obtained from the fusion of NS-1 myeloma cells and spleen cells of the immunized mice. Monoclonal antibodies (MAbs) from 24 of the hybrids exhibited immunobinding to 2B6 as determined by ELISA. One of the MAbs, 49-10-20, showed a strong immunoblotting activity and was highly inhibitory to 2B6 enzyme activity. MAb 49-10-20 inhibited cDNA-expressed 2B6-catalyzed metabolism of diazepam, phenanthrene, 7-ethoxycoumarin, and testosterone by 90-91%. MAb 49-10-20 showed extremely high specificity for 2B6 and did not bind to 17 other human and rodent P450s or inhibit the metabolism of phenanthrene catalyzed by human 1A2, 2A6, 2C8, 2C9, 2D6, 2E1, 3A4, and 3A5. MAb 49-10-20 was used to determine the contribution of 2B6 to the metabolism of phenanthrene and diazepam in human liver. In ten liver samples, MAb 49-10-20 inhibited phenanthrene metabolism variably by a wide range of 8-42% and diazepam demethylation by 1-23%. The degree
of inhibition by the 2B6 specific MAb 49-10-20 defines the contribution of 2B6 to phenanthrene and diazepam metabolism in each human liver. This technique using inhibitory MAb 49-10-20 determines the contribution of 2B6 to the metabolism of its substrates in a human tissue containing multiple P450s. This study is a prototype for the use of specific and highly inhibitory MAbs to determine individual P450 function.