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A neuroprotective agent for ischemia-reperfusion damage, KR-31378, in human plasma and urine and in rat tissue homogenates. The method involved deproteinization of the the biological samples with 0.5 volumes of saturated Ba(OH)2, 0.5 volumes of 0.04 M ZnSO4 and 1 volume of acetonitrile. A 80-ml aliqout of the supernatant was injected onto a reversed-phase C18 column. The mobile phase, 50 mM triethylamine acetate : acetonitrile : tetrahydrofuran (65:30:5, v/v/ A high-performance liquid chromatographic method was developed for the determination of a v), was run at a flow rate of 1.0 ml/min. The column effluent was mornitored by a ultraviolet detector set at 310 nm. The retention time of KR-31378 was approximately 6.5 min. The detection limits of KR-31378 in human plasma and urine and rat tissue homogenates were 0.2, 0.5 and 0.5 mg/ml, respectively. The coefficients of variation (within-day and between-day) were below 13.6% for human plasma and urine and rat homogenates. No interferences from endogenous substances were found.
The blood partition of KR-31378 between plasma and blood cells and the factors influencing the binding of the drug to 4% human serum albumin (HSA) using an equilibrium dialysis technique were evaluated. KR-31378 reached an equilibrium rapidly between plasma and blood cells of rabbit blood. The equilibrium plasma/blood cells concentration ratios were independent of initial rabbit blood concentrations of KR-31378, 1, 10 and 50 μg/ml; the values were in the range of 1.42-2.33. It took approximately 12-h jncubation to reach an equilibrium between plasma and isotonic Sfrensen phosphate buffer of Ph 7.4 containing 3% Dextran ('the buffer') compartments. The mean binding of KR-31378 to 4% HSA was dependent on HSA concentrations (the binding values were 25.3, 25.8 was 25.4% at KR-31378 concentrations ranging from 1 to 100μg/ml. Binding of KR-31378 and 32.0% for HSA concentrations of 2, 4 and 5%, respectively), incubation temperature (the binding values were 48.8, 29.0 and 25.8% for 4, 22, and 37°C, respectively), isotonic Sfrensen phosphate buffer of Ph 7.4 containing 3% Dextran ('the buffer') pHs (the binding values were 17.7, 20.6, 22.8, 25.6 and 29.5% for buffer pHs of 5.8, 6.4, 7.0, 7.4 and 8.0, respectively) and alpha-1-acid glycoprotein (AAG) concentrations (the binding value were 25.6, 29.9, 34.4 and 50.3% for AAG concentrations of 0, 0.08, 0.16 and 0.32%, respectively).

[PE2-3] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Identification of the cytochrome P450 isoenzymes involved in the metabolism of KR-60436 in the human liver microsomes

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The metabolism of KR-60436, a new proton pump inhibitor, has been studied in incubations with human liver microsomes and purified bacterial recombinant cytochrome P450 (CYP) isoforms using liquid chromatography-mass spectrometry.
In the in vitro studies with rat and human liver microsomes, KR-60436 was extensively metabolized to produce at least seven metabolites, which exhibited different H+/K+ ATPase inhibitory activities. Results obtained by using chemical inhibitors or antibodies selectively active against specific CYPs and purified bacterial recombinant CYP isoforms provide a direct evidence for the involvement of CYP3A4, CYP2C9 and CYP2D6 in KR-60436 oxidative metabolism. KR-60436 inhibited CYP1A2-, CYP2E1- and CYP2C9-catalyzed activity, while CYP3A4-related activity was unaffected.

[PE2-4] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]