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CYTOCHROME P-450 DEPENDENT METABOLISM OF L-DEPRENYL BRAIN AND LIVER FROM DIFFERENT ANIMALS

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l-Deprenyl (LD) is a selective monoamine oxidase B (MAO B) inhibitor used in association with l-dopa in the treatment of Parkinson's desease (PD). Several studies, suggested that l-deprenyl possesses neurorescuing/antiapoptotic effects indipendent of its MAO B inhibitory properties. Furthermore this effects seems to be releated to its cytochrome P450 (CYP)-dependent metabolism. LD is metabolized to l-metamphetamine (MA) and l-nordeprenyl (ND), which are subsequently transformed to l-amphetamine. ND has been indicated to posses an antiapoptotic and neuroprotective activities, while l-amphetamine and MA, either if are potent inhibitor of dopamine re-uptake (Magyar et al.,1998), seem to contrast these effects. The superimposition of pharmacological properties of l-deprenyl with those of its metabolites, underline the importance to investigate on the CYP-dependent metabolism of the drug. l-Deprenyl metabolism by liver and brain microsomal preparations obtained from African green monkeys and C57BL mice, animal models extensively used in Parkinson'disease studies, were investigated. Moreover, in order to fill up a gap of knowledge, the characterisation of CYP system in this monkey strain was carried out. Microsomes from African green monkey livers were analyzed for the constitutive expression of P450, cytochrome b_5, P450-reductase and several monoxygenase activities. Levels of, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, benzoyloxyresorufin O-deakylase, coumarin and p-nitrophenol hydroxylases, erythromycin demethylase, dextromethorphan O-demethylase and testosterone hydroxylase activities were found similar to those reported for the cynomolgus or rhesus monkey liver microsomes. Western-blot analysis of both African green and cynomolgus monkey revealed the expression of constitutive proteins immunorelated to P450 1A, 2A, 2B, 2C, 2D, 2E and 3A subfamilies. The use of anti-rat 2C11 antibody showed in both species two immunoreactive protein bands provided with slightly different molecular weights. When l-deprenyl, was used as a substrate for African green monkey liver microsomes, two oxidative N-dealkylation reactions leading to l-methamphetamine and, subsequently, to l-nordeprenyl were observed; they were characterised by a high and a low affinity component. For l-methamphetamine formation, the apparent K_{m1} and K_{m2} were 1.07 ±0.01 and 350±2.7 µM and V_{max1} and V_{max2} were 4.70±0.01 and 8.9±0.02 nmol/min/mg protein, respectively. For l-nordeprenyl formation, the apparent K_{m1} and K_{m2} were 0.96±0.05 and 168±15 µM and V_{max1} and V_{max2} were 3.34±0.02 and 3.91±0.02 nmol/min/mg protein, respectively. At 15 µM l-deprenyl both ketoconazole and 8-methoxypsoralen inhibited l-methamphetamine formation with 5.1 and 8.1 µM IC_{50} and that of l-nordeprenyl with 3.3 and 38.8 µM IC_{50} values, respectively, indicating that P450 3A and P450 2A were involved in both reactions. At high l-deprenyl concentrations, however, also α-naphthoflavone and quinidine inhibited effectively both reactions, indicating the involvement of P450 1A and 2D subfamilies.

While, in contrast with those observed in monkey, the formation of both MA and ND, in mouse liver microsomes, follows a monophasic Michaelis-Menten kinetic and different isozymes seem to be involved in l-deprenyl-CYP-dependent metabolism (Valoti et al., 2000). Kinetic analysis, performed in monkey brain microsomes, has shown that the major product of LD metabolism was l-metamphetamine both in cortex and in striatum. The values of Vmax of formation of MA was 28.60 ± 1.7 pmol x min^{-1}x mg $prot^{-1}$ and 9.2 ± 0.8 pmol x min^{-1} x mg $prot^{-1}$ in cortex and striatum respectively , while the Vmax of formation of ND was of one order of magnitude lower both in cortex and striatum microsomes (6.5 ± 0.5 and 0.94 ± 0.05 pmolx min^{-1} xmg $prot^{-1}$ respectively). The Km of formation of MA were similar in the two cerebral areas investigated (67.8 ± 1.0 µM and 72.0 ± 1.6 µM in the cortex and striatum respectively), also the Km of formation of ND were similar (21.3 ± 3.2 µM and 27.3 ± 4.0 µM in the cortex and striatum respectively).
Kinetic analysis performed in C57BL mice brain microsomal preparations showed that MA formation was similar to those found in whole monkey brain microsomes (Km= 53.6 ± 2.9µM; Vmax= 33.9 ± 0.4 pmol x min^{-1} x mg $prot^{-1}$), while the formation of l-nordeprenyl was not detectable.
In order to clarify the involvment of different CYP isozymes in monkey brain microsomes, LD was incubated in presence of selective inhibitors: 4-methylpyrazole, ketoconazole, and 8-metoxypsoralen. Ketoconazole and 8-metoxypsoralen showed a concentration-dependent inhibition of MA formation with IC50 of $8.9x10^{-6}$M, $2.9x10^{-6}$M. On the contrary 4-methylpyrazole, at the highest concentration used

(100µM), promoted negligible inhibition on MA formation. Moreover 8-metoxypsoralen and 4-methylpyrazole were not able to inhibit N-demethylation rate, while ketoconazole showed a slight inhibition in the formation of l-nordeprenyl with a IC50 of $3.04x10^{-5}$ M.
The experiments performed suggest that MA is the major product of LD metabolism in monkey and C57BL mice brain. Furthermore, different CYP isozymes are involved in LD metabolism and in particular 3A4 and 2A6 seem to be the major responsibles of MA formation in monkey brain, according with the results observed in monkey liver.
In conclusion, metabolic disposition of l-deprenyl by monkey and mouse liver microsomes is very efficient, thus indicating that CYP-dependent metabolism is relevant and could contribute to neuroprotection. A different metabolic pathway seems to attend l-deprenyl metabolism in mouse liver microsomes. In this case CYP2E1 seems the main isozymes involved in both N-dealkylation reactions in C57BL mice liver. The evidence that CYP2E1 is not involved in monkey liver l-deprenyl CYP-dependent metabolism, underline the differences between species in l-deprenyl metabolism and the dangers of attempting to extrapolate results.

References

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