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Blood–brain barrier penetration and pharmacokinetics of amitriptyline and its metabolites in p-glycoprotein (abcb1ab) knock-out mice and controls

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Abstract

In earlier studies with P-gp (abcb1) knock-out mice, we showed that P-gp exports the antidepressants citalopram, paroxetine, venlafaxine and amitriptyline and its metabolites across the blood–brain barrier, thereby reducing cerebral bioavailability of some substances up to 9 times. The present study investigated the pharmacokinetics of amitriptyline and whether abcb1ab double knock-out mice metabolize amitriptyline and its metabolites differently. P-gp knock-out mice and controls received a s.c. injection of 10 μg amitriptyline/g of body weight. The animals were sacrificed after 30, 60, 120 and 240 min and concentrations of amitriptyline and its metabolites were measured with HPLC in brain, plasma, liver, kidney, spleen, lung, muscle, fat and ovaries. Cerebral concentrations of amitriptyline and its metabolites were higher in P-gp-deficient mice compared to controls. No significant group effect was found for spleen, liver, lung, kidney and fat tissue. The results of our study indicate that amitriptyline and its metabolites are substrates of P-gp. Overall pharmacokinetics between knock-outs and controls were very similar. This confirms the validity of the P-gp knock-out model and allows for a continued research of the interactions between P-gp, the blood–brain barrier and CNS substances such as antidepressants, neuroleptics and others.

Keywords: P-glycoprotein; abcb1; Blood–brain barrier; Amitriptyline; Nortriptyline; Knock-out mice

1. Objectives of the study

P-glycoprotein (P-gp) is a 170-kDa ATP-dependent drug transport protein, located in the apical membrane of endothelial cells. Utilizing ATP hydrolysis as an energy source, it exports molecules which attempt to pass through the cell membrane from the outside to the inside, protecting cells from toxins and a wide range of substances. P-gp belongs to a large and growing (Eisenblätter and Galla, 2002; Eisenblätter et al., 2003) group of transmembrane transporters, which are increasingly recognized as an important part of the blood–brain and blood–CSF barriers (Borst et al., 2000; Lee et al., 2001; Demeule et al., 2002; Bendayan et al., 2002). P-glycoprotein is expressed and was first discovered in multiple drug resistant (MDR) cancer cells, but can also be found in normal tissue. This includes the apical membrane of intestinal epithelial cells (Mukhopadhyay et al., 1988), the biliary canalicular membrane of hepatocytes, and the lumenal membrane of proximal tubular epithelial cells in the kidney (Thiebaut et al., 1989; Demeule et al., 2001). High levels of ABCB1 (formerly called MDR), P-glycoprotein have been found in the luminal membrane of the endothelial cells that line small blood capillaries and form the blood–brain, blood–CSF and blood–testis

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barriers (Cordon-Cardo et al., 1989; Wijnholds et al., 2000; Tamai and Tsuji, 2000; Fromm, 2000), reflecting the important role P-gp plays in the function of the blood–brain barrier.

In mice, P-gp is encoded by the abcb1a (formerly called mdr1a) and the abcb1b (formerly called mdr1b) gene (Gottesman et al., 1995); the overall distribution in mice tissue overlaps well with the single ABCB1 gene in humans. In humans, P-gp is encoded by the multiple drug resistance (ABCB1) gene, shares many features with numerous bacterial and eucaryotic ATP-binding cassette (ABC) transport proteins and is a member of a phylogenetically highly conserved superfamily of transport proteins (Devault and Gros, 1990; Borst et al., 2000; Kerb et al., 2001).

Since 1994 knock-out mice which lack the abcb1a gene for P-gp have been available (Schinkel et al., 1994), and have enhanced the understanding of the importance and function of P-gp. Recent work has shown that beside the important role P-gp has in the maintenance of the blood–brain barrier, it also plays a protective role in the inner ear (Zhang et al., 2000) and in the placenta, where it limits fetal penetration of various potentially harmful or therapeutic substances (Smit et al., 1999).

Earlier it was shown that amitriptyline and its metabolites are substrates of P-gp (Uhr et al., 2000) and actively exported out of the brain back into the extracellular space. This is in accordance with the observation that St. John's wort, an inhibitor of P-gp, decreases the blood concentrations of amitriptyline (Johne et al., 2002; Zhou et al., 2004). One hour after a one-time intraperitoneal (i.p.) administration, brain concentrations of amitriptyline and its metabolites are significantly higher in abacb1a knock-out mice compared to controls (Uhr et al., 2000). After repeated subcutaneous (s.c.) administration over 10 days, this trend continues for the metabolites of amitriptyline, and the CNS concentrations are up to 9-fold higher in abcb1ab knock-out mice. Amitriptyline itself ceased to differ significantly four hours after the last injection (Grauer and Uhr, 2004).

In related to one-dose studies, we showed that citalopram, trimipramine, doxepin, venlafaxine and paroxetine are also substrates of P-gp, and that the concentration of these substances in the brains of knock-out mice is up to three times higher (Uhr and Grauer, 2003; Uhr et al., 2003). However, as studies on fluoxetine (Uhr et al., 2000), melperone (Uhr et al., 2004) and mirtazapine (Uhr et al., 2003) illustrated, not all CNS drugs are P-gp substrates. Recently, the relationship between P-gp and thirty-two structurally diverse drugs used to treat various conditions of the CNS has been investigated (Doran et al., 2005). Furthermore, it has been shown that not only CNS drugs can be substrates of P-gp, but also endogenous glucocorticoid hormones such as cortisol, corticosterone, aldosterone and progesterone (Uhr et al., 2002).

In general, it is somewhat unexpected that substances which are effective CNS drugs are exported by P-gp to such an extent. Furthermore, it is surprising that, as in the case of amitriptyline, the metabolites of a drug continue to accumulate in the brain over 10 days, whereas the parent drug amitriptyline reaches the same cerebral concentration in knock-outs and controls.

One question that arises is: do P-gp knock-out mice metabolize amitriptyline differently? This would explain some of the short-term differences in CNS concentrations between knock-outs and controls and call into question the validity of the animal model used. If P-gp knock-out mice metabolize drugs differently, then conclusions based on experiments with P-gp knock-out mice have to be drawn with great care. The objective of the study was to determine whether knock-out mice and controls differ in their pharmacokinetics with respect to amitriptyline.

2. Materials and methods

2.1. Animals

Female abcb1ab (−/−) mice ($n = 24$) and FVB/N wild-type mice ($n = 24$) were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 07:00), with food and water ad libitum. The age of the animals used was between 19 and 24 weeks. The average weight of abcb1ab (−/−) mice was 27.5 ± 0.5 g, of controls 23.6 ± 0.5 g. abcb1ab double knock-out mice, originally created by A. Schinkel by sequential gene targeting in 129/Ola E14 embryonic stem cells (Schinkel et al., 1997) and backcrossed seven times (N7) to FVB/N from the C57BL/6 × 129 chimera. FVB/N wild-type mice were received from Taconic (Germantown, USA; FVB/Tac-[KO]Pgy2 N7). A homozygous colony is maintained at the Max Planck Institute of Psychiatry on the N7 FVB/N background through intercrossing of homozygous mice.

All animal experiments were carried out in accordance with the Animal Rights Act of the State of Bavaria, which regulates the use and treatment of experimental animals. The supervising animal care coordinator of the State of Bavaria agreed with all housing and experimental procedures. P-gp knock-out mice and controls received a single s.c. injection of 10 μg amitriptyline/g of body weight. The animals were sacrificed after 30, 60, 120 and 240 min and concentrations of amitriptyline and metabolites were measured with HPLC (as described before, Uhr et al., 2000) in brain, plasma, liver, kidney, spleen, lung, muscle, fat and ovaries.

As a nominal level of significance $\alpha = 0.05$ was accepted and corrected (reduced according to the Bonferroni procedure) for all a posteriori tests (multivariate *F*-tests