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The anti-Parkinson drug budipine is exported actively out of the brain by P-glycoprotein in mice

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Abstract

P-glycoprotein, a product of the ABCB1 gene, is a plasma membrane transporter that exports certain drugs as well as endogenous substances against a concentration gradient in the intestines, kidney and testes. It also constitutes an important part of the blood–brain barrier, where it exports its substrates out of the brain back into the circulation. To investigate whether the uptake of the anti-Parkinson drug budipine into the brain is mediated by P-glycoprotein, abcb1ab(−/−) double knock-out mice and wild-type control mice received budipine continuously over 11 days via implanted osmotic infusion pumps at the rate of 30 ug over 24 h. Concentrations of the drug in plasma, brain, and organs were measured with HPLC. Budipine concentrations in the abcb1ab knock-out animals were 3.1 times higher than in control mice. This study confirms the important role P-gp plays at the blood–brain barrier and shows that budipine is a substrate of P-gp.

Keywords: Multiple drug resistance; Knock-out mice; P-glycoprotein; Blood–brain barrier; Budipine; abcb1

Budipine is an anti-Parkinson drug which was first synthesized in 1979 and has been in clinical use since the mid-1980s. The clinical advantages of budipine are: its good effect on tremor, relatively few side effects and usefulness in combination therapy with L-DOPA. After a period of decreasing use, it has again gained popularity [9]. The mechanism of action is increasingly well understood and has been well described [1]. Besides being a *N*-methyl-D-aspartate (NMDA) antagonist [7], budipine has indirect dopaminergic effects through an improved dopamine release, the inhibition of monoamine oxidase type B (MAO-B) and through the stimulation of the rate-limiting enzyme in dopamine production, aromatic L-amino acid decarboxylase (AADC) [2]. However, very little is known about the interaction between budipine and the blood–brain barrier (BBB), the concentrations of budipine in the brain and CSF and its pharmacokinetics after longer administration.

ABCB1 P-glycoprotein (P-gp) is a 1280 amino acid, glycosylated plasma membrane protein. It can actively transport its substrates against a concentration gradient utilizing ATP hydrolysis as an energy source. P-gp is expressed in the lumenal membrane of the endothelial cells that line capillaries forming the blood–brain barrier [11]. The transported substrates for P-glycoprotein include many classes of drugs [17,13].

In an earlier study [18], we were able to show that concentrations of amitriptyline and its metabolites in the brain were two to four times higher in P-glycoprotein knock-out mice, showing that amitriptyline is a substrate of P-gp, actively exporting amitriptyline out of the cell. Further studies revealed that the antidepressants trimipramine, citalopram, doxepin, venlafaxine and paroxetine are P-gp substrates [15,16]. P-gp is therefore an important component of the BBB and has an impact on the bioavailability of certain drugs and endogenous transmitters in the brain. We hypothesize that P-gp can be an obstacle to the successful treatment of Parkinson's disease and other CNS disorders and that interactions between CNS drugs and P-gp can be of clinical relevance in the realms

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of therapeutic efficacy and side effects. Any physician who administers CNS drugs has experienced that it is impossible to predict the wide range of possible outcomes, including non-response and side effects, when therapy is initiated. Purpose of the study was to investigate whether a frequently used neurological drug is a substrate of P-gp, and therefore maybe subject to varying uptake in the brain with a resulting difference in bioavailability.

Budipine was obtained from Lundbeck (Copenhagen, Denmark). Male abcb1ab(−/−) mice ($n = 8$, weight 29.0 ± 0.9 g) and FVB/N wild-type mice ($n = 8$, weight 28.0 ± 0.5 g) were housed individually and maintained on a 12/12 h light/dark cycle (lights on at 07:00 a.m.), with food and water ad libitum. abcb1ab double knock-out mice, originally created by A. Schinkel by sequential gene targeting in 129/Ola E14 embryonic [12] and backcrossed seven times (N7) to FVB/N from the C57BL/6 × 129 chimera, and 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. Standards of animal experiments complied with German animal protection law.

Budipine dissolved in 0.9% sodium chloride and 0.5% ethanol was administered s.c. in the nape of the neck through surgically implanted osmotic infusion pumps (Alzet® microosmotic pump, Alza corporation, Palo Alto, USA), which continuously delivered 30 ug of budipine over 24 h. After 11 days, the mice were anesthetized with halothane and decapitated. Extraction procedure was performed as described before [16]. The dissected organs were homogenized and liquid–liquid extraction was carried out with *n*-hexane in the first step and phosphoric acid in the second step. The extraction recoveries were >90%.

HPLC measurements were performed as described before [16], using protriptyline 10 μg/mL as the internal standard solution, the procedure was modified for budipine with a mobile phase gradient of 25–75%, concentrations were measured with UV absorption at 214 nm and with fluorescence at 220/290 nm.

Statistical analysis was carried out by the Statistics Department of the Max Planck Institute of Psychiatry. Significance

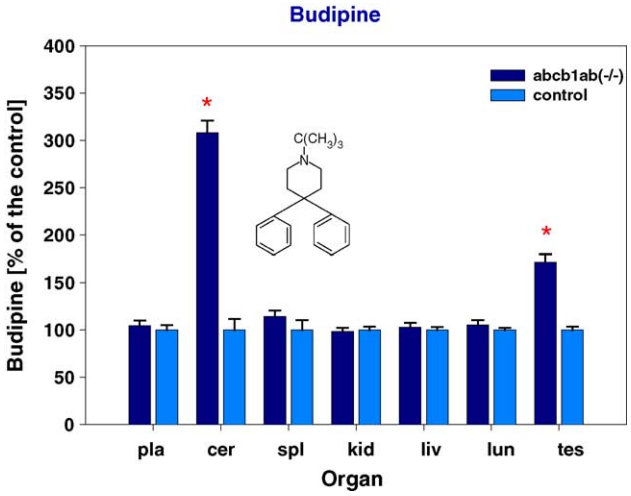

Fig. 1. Plasma and organ concentration of budipine after 11 days of continuous s.c. administration through osmotic pumps with a dose of 30 ug/24 h. Values are shown as mean ± S.E.M. and as percentage of controls, which are at 100%. The asterisk "*" marks significant differences between knock-outs and controls (univariate *F*-test in MANOVA, $p < 0.05$).

was tested by one-factorial multivariate analysis of variance (MANOVAs). Univariate *F*-tests followed to identify the variables whose differences between the two groups contributed significantly to the global group effect. As a nominal level of significance $\alpha = 0.05$ was accepted and corrected (reduced according to the Bonferroni procedure) for all a posteriori tests (univariate *F*-tests) in order to keep the type I error less than or equal to 0.05.

After an 11-day continuous administration, the cerebrum concentration for budipine was 3.1 times higher in knock-out mice compared to wild-type controls (Table 1 and Fig. 1). Analysis of variance revealed a significant group effect on the budipine concentrations (Wilks multivariate test of significance; effect of group: $F(7,7) = 62.1$; significance of $F < 0.001$). There were no significant differences in the budipine concentrations in plasma, spleen, kidney and liver. Concentrations in the testes were 1.7 times higher, which was also significant.

The present study shows that the anti-Parkinson agent budipine is a substrate of P-glycoprotein (P-gp) and is

Table 1
Absolute plasma and organ concentration of budipine after 11 days of continuous s.c. administration through osmotic pumps with a dose of 30 ug/24 h

	abcb1ab(−/−)	S.E.M.	Controls	S.E.M.	abcb1ab(−/−)/(+/+)	Significance
Budipine (ng/g or ng/ml)						
Plasma	50.26	2.65	48.09	2.42	1.0	ns
Cerebrum	*229.53*	*9.57*	*74.51*	*8.62*	*3.1*	*
Spleen	318.92	16.77	278.59	29.47	1.1	ns
Kidney	636.68	23.84	645.76	23.76	1.0	ns
Liver	450.10	20.58	437.65	13.02	1.0	ns
Lung	660.09	32.87	627.13	14.33	1.1	ns
Testes	659.58	31.03	384.33	14.27	1.7	*

Group effect: $F(7,7) = 62.1$; significance of $F < 0.001$. Values are shown as mean ± S.E.M. The asterisk "*" marks significant differences between knock-outs and controls (univariate *F*-test in MANOVA, $p < 0.05$).