**The Influence of P-glycoprotein Inhibition on Imipramine Transport across the Blood-Brain Barrier: Microdialysis Studies in the Conscious Freely Moving Rat**

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Recent studies indicate that antidepressant efflux by the multidrug resistance transporter P-glycoprotein (P-gp) at the blood-brain barrier (BBB) may contribute to treatment resistant depression (TRD) by limiting intracerebral antidepressant concentrations [1]. In addition, anecdotal evidence suggests that adjunctive treatment with the P-gp inhibitor verapamil may improve the clinical outcome in TRD [2]. Therefore, the present study aimed to investigate the influence of P-gp inhibition on the transport of the tricyclic antidepressant imipramine (IMI) and its active metabolite desipramine (DMI) across the BBB.

Intracerebral microdialysis [3] was used to monitor levels of IMI and DMI in the prefrontal cortex following intravenous IMI administration in male Sprague Dawley rats (255-290 g), with or without pre-treatment with one of the P-gp inhibitors verapamil or cyclosporin A (CsA). Indwelling catheters were surgically placed into the jugular vein and carotid artery of the rats to facilitate intravenous drug administration and blood sampling, respectively. After 16-24 hours post-operative recovery, IMI (5 $mg.kg^{-1}$ i.v.) was administered to all rats. Rats were separated into three groups (n=6 per group): 1. IMI only; 2. IMI+VER: pre-treated with verapamil (20 $mg.kg^{-1}$ i.p.) 90 minutes before IMI administration; 3. IMI+CsA: pre-treated with CsA (25 $mg.kg^{-1}$ i.v.) 30 minutes before IMI administration. IMI and DMI concentrations in plasma and microdialysis samples (dialysates) were determined over a 4 hour period post-IMI administration by HPLC with electrochemical detection. Plasma samples were taken before IMI administration (blank) and at 5, 15, 30, 60, 120, 180 and 240 minutes post-IMI administration. Dialysate samples were collected continually at 20 minute intervals throughout the sampling period. Statistical analysis of results was carried out using one-way ANOVA, with 2-way Dunnett's post-hoc test where appropriate. All data are presented as mean (± SEM).

Pre-treatment with either verapamil or CsA resulted in significant increases in dialysate IMI concentrations ($p \leq 0.05$) relative to the IMI only group, without altering IMI levels in plasma. The dialysate IMI area under the concentration-time curve (AUC; unit: $ng.ml^{-1}.min$) was increased from 1322 (± 98) in the IMI only group to 1802 (± 144) and 2108 (± 169) in the IMI+VERAP and IMI+CsA groups respectively. The mean dialysate:plasma IMI AUC ratio, which gives an indication of BBB transport, was significantly elevated by 84% in the IMI+CsA group relative to the IMI only group (0.0274 ± 0.0038 vs 0.0149 ± 0.0024; $p<0.05$), while the 44% increase observed in the IMI+VERAP group compared to the IMI only group did not reach statistical significance (0.0215 ± 0.0028 vs 0.0149 ± 0.0024; $p>0.05$). Furthermore, pre-treatment with verapamil, but not CsA, led to a significant elevation in plasma and brain levels of DMI relative to the IMI only group ($p < 0.001$). Plasma DMI AUC values were 46050 (± 4984) in the IMI+VERAP group, compared to 11137 (± 2393) in the IMI only group. DMI dialysate levels could only be determined in the IMI+VERAP group (158.4 ± 14), as dialysate concentrations were below the limit of quantification in the other two groups.

The present study demonstrates that P-gp inhibition can enhance intracerebral IMI concentrations. Furthermore, this study highlights a potentially important pharmacokinetic interaction between IMI and verapamil, whereby co-administration of verapamil leads to significantly elevated levels of the active IMI metabolite, DMI, both in plasma and the brain. Taken together, these findings may help to explain reports of a beneficial response to adjunctive therapy with verapamil in TRD, and highlight a potential therapeutic role for P-gp inhibitors in the clinical management of TRD.

[1] O'Brien FE *et al.* (2012). Br J Pharmacol 165(2): 289-312.
[2] Clarke G, *et al.* (2009). Hum Psychopharmacol 24: 217-223.
[3] de Lange ECM *et al.* (2000). Adv Drug Deliver Rev 45: 125-148.