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Carbamazepine Is Not A Substrate For ABCC2

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Introduction: Carbamazepine (CBZ) is a widely used first-line drug for the treatment of epilepsy. About 30% of all patients, however, fail adequate seizure control. A number of clinical studies have suggested an association between CBZ treatment resistance and variants of the drug efflux transporter ATP-binding cassette transporter C2 (ABCC2, MRP2). In a study with Caucasian patients, a higher probability for treatment response was observed for the 1249G>A variant of ABCC2 (Ufer et al., 2011). In an Indian population, seizure control in women was related to variants in the ABCC2 promotor region (-1549G>A and -1019A>G, Grover et al., 2012). Interestingly, the 1249G>A variant was also reported to be related to CBZ adverse drug reactions in a Korean case-control study (Kim et al., 2010). Functional studies with vesicles overexpressing ABCC2 WT and the 1249G>A variant (valine substituted by isoleucine at position 417) revealed that CBZ can inhibit efflux of the ABCC2 substrate 5,6-carboxyfluorescein (CF) in WT vesicles but not in the variant (Kim et al., 2010). The authors concluded that CBZ might be a substrate for ABCC2 and that the variant is affecting CBZ efflux leading to better treatment responses. However, this indirect approach has to be interpreted with caution, especially since a different *in vitro* study could not confirm that CBZ was a substrate of ABCC2 (Luna-Tortos et al., 2010). This question thus remains controversial and requires further investigation to assess the potential clinical importance of ABCC2 in CBZ treatment failure.

Methods: *In vitro* transporter efflux assays were performed in two different cell lines, a human fibrosarcoma cell line stably transfected with ABCC2 tagged to EGFP (Rht14-10 MRP2-EGFP, Arlanov et al., 2012), and Madin Darby canine kidney cell line II (MDCKII) stably transfected with ABCC2 (kind gift from Prof. Dr. P. Borst, Netherland Cancer Institute, Amsterdam, NL). The assay was performed as described before (Arlanov et al., 2012). 5µM CellTracker™ Green 5-Chloromethylfluorescein diacetate (CMFDA) was applied as a positive control. Tritium labelled CBZ (3H-CBZ) was taken as a tracer and mixed with non-labelled CBZ to give a final concentration of 5µM.

Vesicle uptake experiments were undertaken for 20 minutes in inside-out vesicles prepared from Rht14-10 MRP2-EGFP and control cells as described previously (Keppler et al., 1998). 50µM 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (CDCF) mixed isomers was applied as a positive control (Pratt et al., 2006). 5µM CBZ was used as in efflux assays.

Results: No difference in CBZ efflux could be observed between the ABCC2 overexpressing cell lines (Rht14-10 and MDCKII) and controls. In addition, vesicle uptake experiments showed no difference between Rht14-10 MRP2-EGFP and control vesicles, either in the presence of adenosine triphosphate (ATP) or adenosine monophosphate (AMP).

Discussion: CBZ is not actively transported by ABCC2 using more robust assays than have been previously employed in the literature. This calls into question the role of ABCC2 in determining resistance to CBZ therapy in epilepsy. Although our data shows that CBZ is not a substrate for ABBC2, we cannot exclude the possibility that it acts as an inhibitor of ABCC2 (Kim et al., 2010).