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RESEARCH PAPER

Exploring the Carbamazepine Interaction with Human Pregnane X Receptor and Effect on ABCC2 Using *in Vitro* and *in Silico* Approach

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ABSTRACT

Purpose Over expression of ATP-binding cassette transporters is considered one of the major reasons for non-responsiveness to antiepileptic drugs. Carbamazepine (CBZ), one of first line antiepileptic drug is known to influence *ABCC2* expression but its exact molecular mechanism is unknown.

Methods We investigated the effect of CBZ on expression of *ABCC2* and pregnane X receptor (PXR) in HepG2 cell line and compared with hyperforin (agonist of PXR) and ketoconazole (antagonist of PXR) through realtime PCR and western blot assay. Involvement of PXR was demonstrated through nuclear translocation and RNA interference and related effect of CBZ on ABCC2 through functional activity assay. Molecular docking and dynamic simulation approach was used to understand the interaction of CBZ with PXR.

Results CBZ and hyperforin increased the PXR and ABCC2 expression whereas reversed when present it in combination with ketoconazole. Experiments confirmed CBZ induced *ABCC2* expression is PXR dependent. Molecular dynamic (MD) simulation and *in vitro* experiment indicated possibility of CBZ to be PXR agonist and PXR residue Gln285 to be important for CBZ-PXR interaction.

Conclusions CBZ alters the functional activity of ABCC2 through PXR, which in turn can interfere with therapy. Mutational analysis of residues revealed the importance of Gln285 in ligand interaction.

KEY WORDS ABCC2 · carbamazepine · molecular dynamics simulation · pregnane xenobiotic receptor

ABBREVIATIONS

ABC	ATP-binding cassette
AEDs	Antiepileptic drugs
CBZ	Carbamazepine
CFDA	Carboxyfluorescein diacetate
MD	Molecular Dynamics
PXR	Pregnane X receptor

INTRODUCTION

Epilepsy is a neurological brain disorder, characterized by recurrent seizures, affecting around 1–2% of population worldwide (1). Its treatment includes use of antiepileptic drugs (AEDs) to control seizures, but 20–30% of patients fail to respond to AED pharmacotherapy, which puts such patients in life threatening situations and is a major clinical problem (2). Over expression of ATP-binding cassette (ABC) transporters is implicated as one of the major reasons for non-responsiveness to AED pharmacotherapy. Various studies have associated ABC transporters such as P-glycoprotein (Pgp, also known as ABCB1), multidrug resistance-associated proteins (MRPs, known as ABCCs), and breast cancer resistance protein (BCRP, known as ABCG2) with antiepileptic drug resistance (3,4). Out of these, ABCC2, also known as multi drug resistance associated protein 2 (MRP2) is especially important because it is located at apical membrane domain of polarized cells which supports its role in terminal phase of detoxification (5,6). International Transporter

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