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Short communication

Upregulation of breast cancer resistance protein and major vault protein in drug resistant epilepsy

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ABSTRACT

Purpose: Identifying factors involved in the development of drug resistant epilepsy (DRE) remains a challenge. Candidate gene studies have shown modulation of resistance to drugs by various multidrug resistance proteins in DRE. However the resistance to drugs in DRE could be more complex and multifactorial involving molecules in different pharmacokinetic processes. In this study for the first time we have analyzed the relative expression of four molecules with different drug resistance mechanisms in two most common DRE pathologies, mesial temporal lobe epilepsy (MTLE) and focal cortical dysplasia (FCD) with respect to each other and also with different non-epileptic controls.
Methods: Brain tissues resected from MTLE (n = 16) and FCD type I and II (n = 12) patients who had undergone surgery were analysed for mRNA levels of multidrug resistance-associated protein 1(*MRP1*), major vault protein (*MVP*), breast cancer resistance protein (*BCRP*), and one drug metabolising enzyme (*UGT1A4*) as compared to non-epileptic controls which were tissues resected from tumor periphery (n = 6) and autopsy tissues (n = 4) by quantitative PCR.
Results: We found significant upregulation of *MVP* and *BCRP* whereas *MRP1* and *UGT1A4* were unaltered in both pathologies. While upregulation of *BCRP* was significantly higher in MTLE (9.34 ± 0.45; $p < 0.05$), upregulation of *MVP* was significantly higher in FCD (2.94 ± 0.65; $p < 0.01$).
Conclusion: We propose that upregulation of *BCRP* and *MVP* is associated with MTLE and FCD and these molecules not only may have the potential to predict pathology specific phenotypes but may also have therapeutic potential as adjunct treatment in these pathologies.

1. Introduction

Despite the availability of new antiepileptic drugs (AEDs), ~20%–30% of people with epilepsy will not be seizure free and are said to have drug resistant epilepsy (DRE) [1]. DRE imposes serious threats to a patient's life including neuropsychological illness, psychiatric and social impairment, reduced marriage rates and decreased lifespan [2]. It is these patients who require a great deal of time and effort for effective treatment. There is also an economic burden on these patients with epilepsies (PWE) [2]. For better management of DRE, it is crucial to understand the exact pathogenesis of this neurological disorder. DRE is likely to be a multifactorial process including genetic factors, disease-related factors and factors resultant from treatment with AEDs [1,3]. There are several reports showing associations between many genetic variations and clinical drug resistance; however these associations are conflicting and are not equivocally replicated [4–6]. Several mechanisms of drug resistance have been proposed such as dysregulation of multidrug transporters (MDTs), reduced drug-target sensitivity, increased neuronal apoptosis, cytoskeletal alterations and reorganization of neuronal networks but the exact mechanism underlying drug resistance remains an enigma [3,7,8]. Altered expression of transporters and metabolic enzymes in blood-brain barrier (BBB) as well as barrier leakage have been linked to AED resistance and seizure genesis, respectively [8].

Abbreviations: DRE, drug resistant epilepsy; PWE, patients with epilepsies; AEDs, anti-epileptic drugs; MTLE, mesial temporal lobe epilepsy; FCD, focal cortical dysplasia; BCRP, breast cancer resistance protein; MVP, major vault protein; MDT, smultidrug transporters; BBB, blood brain barrier; UGT1A4, UDP-glucuronosyl-transferase; qPCR, quantitative PCR.

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Various multidrug resistance proteins such as multidrug resistance gene-1 (MDR1), MRP2 and MRP5 have been shown to be over expressed in animal models of epilepsy as well as in brain tissue resected from DRE patients [9–13]. Prior investigations have shown involvement of MRP1, BCRP, MVP and UGT1A4 in drug resistance in various neurological diseases as well as in cancer yet very few reports are available on their contribution to DRE [13–16]. MRP1 is a specific organic anion transporter and can cause efflux of antitumor drugs and some AEDs namely phenobarbital, carbamazepine, phenytoin, sodium valproate [12]. MVP is a lung infection resistance-related protein and is shown to play role in the vesicular transport of several compounds. MVP is non-specific in nature as a transporter and may affect the response to AEDs, by changing the subcellular compartmentalization of drugs [14]. UGT1A4 gene encodes a UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway and is shown to metabolize AEDs [15]. UGT1A4 is induced by carbamazepine and phenytoin and inhibited by sodium valproate. Role of UGT1A4 has so far not been clearly validated in DRE. BCRP is an ATP-dependent drug efflux transporter with a wide range of substrates, such as antitumor and few AEDs like carbamazepine, oxcarbazepine [16]. Reports related to the contribution of BCRP in AED resistance are contradictory. Some studies found no upregulation of BCRP in human epileptogenic brain tissue and no evidence for BCRP mediated AED transport in vitro, but other studies reported upregulation of BCRP expression in the microvasculature of epileptogenic brain tumors and in chronic epilepsy animal models [13,14]. Alterations in the expression level of transporters in the brain tissues of DRE patients is clinically relevant and need not to be extrapolated, unlike animal models. In order to understand the molecular mechanisms associated with DRE and identify pathology specific alterations with diagnostic and therapeutic potentials, we conducted this study. We analysed the possible association of four molecules with different drug resistance mechanisms, *MRP1*, *MVP*, *UGT1A4* with equivocal and *BCRP* with previously published contradictory reports, in two common DRE pathologies, mesial temporal lobe epilepsy (MTLE) and focal cortical dysplasia (FCD).

2. Materials and Methods

2.1. Ethical Statement

This study was reviewed and approved by Institutional Ethics Committee (IEC), All India Institute of Medical Sciences, New Delhi, India and Institutional Human Ethics (IHE), National Brain Research Centre, Manesar, Haryana, India, before the study began.

2.2. Patients

Patients who were diagnosed with DRE due to MTLE and FCD (Type I and II) and underwent surgery were included in this study (Table 1). Patients with dual pathology were not included in this

Table 1
Clinical characteristics of Patients.

PATIENT NAME	AGE (Years)/SEX	PATHOLOGY/COD	AEDs
M1	18/M	MTLE	CBZ, CLN, VPA
M2	24/F	MTLE	CLO, LEV, OXC
M3	26/F	MTLE	CBZ, CLO, ZNS
M4	39/M	MTLE	CLO, LEV, PBT, PHT
M5	15/M	MTLE	CLO, PHT
M6	15/M	MTLE	LEV, VPA, LTG, ZNS, CLO
M7	37/F	MTLE	LTG, VPA, CLO
M8	11/F	MTLE	CBZ, CLO
M9	20/M	MTLE	CLO, VPA
M10	36/M	MTLE	CBZ, CLO, LCS, LEV
M11	9/M	MTLE	PHT, CBZ
M12	27/F	MTLE	LEV, CBZ
M13	6/M	MTLE	CLO, LEV, PHT, VPA, CLN
M14	22/M	MTLE	CBZ, CLO, TPR
M15	15/M	MTLE	CLO, LCS, LTG, LEV
M16	20/F	MTLE	CLO, LCS, LEV
F1	12/F	FCD Type Ia	CLO, PHT, LEV
F2	24/F	FCD Type Ia	CBZ, LEV, TPR
F3	2/M	FCD Type Ia	CLN, VPA, LEV, VBN, ZNS
F4	6/M	FCD Type Ib	VPA, CLO
F5	11/F	FCD Type Ib	CBZ, CLO, LEV
F6	19/M	FCD Type Ib	CLO, CLN, LTG, LEV, VPA
F7	12/M	FCD Type Ic	LTG, VPA, TPR
F8	15/M	FCD Type Ic	VPA, LEV
F9	13/M	FCD Type IIa	CBZ, CLO
F10	15/M	FCD Type IIa	OXC, CLO, LCS
F11	28/F	FCD Type IIb	CLO, LEV
F12	34/M	FCD Type IIb	CLO, PHT, VPA
C1	26/F	Temporal-occipital tumour	–
C2	34/F	Right medial frontal low grade glioma	–
C3	18/M	Right frontal low grade glioma	–
C4	26/F	Temporal-occipital tumour	–
C5	35/M	Right Temporopartial Glioma	–
C6	30/M	Choroid Glioma	–
A1	25/M	Pelvic Injury	–
A2	18/F	Pelvic and lower limb injury	–
A3	16/M	Abdominal injury	–
A4	18/M	Head and abdominal injury	–

COD- Cause of death, M- MTLE, F- FCD, C- Control, A- Autopsy, CBZ- Carbamazepine, CLO –Clobazam, CLN- Clonazepam, DZP- diazepam, LEV- Levetiracetam, LTG –Lamotrigine, LZP- Lorazepam, OXC- Oxcarbazepine, PBT- Phenobarbital, PHT- Phenytoin, TPR –Topiramate; VPA-Valproinic acid, LCS- Lacosamide, ZNS- Zonisamide, VBN-Vigabatrin.

study. The excision was based on clinical evaluations and pathology was verified by histopathologic examinations as described previously [4]. As there are no "ideal" or acceptable non-epilepsy controls for studies involving epilepsy surgery, we used two different sets of controls in our study. Cortical tissues resected from tumour periphery (TP) during brain tumour surgery of seizure-free patients, hippocampus (H) and frontal lobe (FL) tissues from autopsy patients without any brain pathology were used as non-epileptic controls [4]. The clinical characteristics of the patients are presented in Table 1. Majority of the AEDs administered to our patients carbamazapine, clobazam, phenytoin, and sodium valproate are transported by MRP1, BCRP and metabolized by UGT1A4 (Table 1). For tissue preparation ~5 mm thick hippocampal slices or cortical tissues were stored in RNAlater stabilization reagent (Qiagen) in order to prevent degradation of RNA. The samples were stored at 4 °C overnight and subsequently frozen and kept at −70 °C until further processing.

2.3. Quantitative PCR (qPCR) methodology and Analysis

Specific primers for *MRP1*, *BCRP*, *UGT1A4*, *MVP* and *HPRT* genes were designed using the Primer-BLAST (Primer3Input, version 0.4.0 and BLAST, available at http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Supplementary Table 1). qPCR was performed in triplicates in an independent set of four frontal lobes (FL1-FL4) and four hippocampus (H1-H4) from autopsy (A), six tumor periphery tissues (TP1-TP6) from tumor/glioma patients (Control: C1-C6), sixteen MTS (M1-M16) and twelve FCD (F1-F12) patients. RNA was extracted, purified and analyzed for purity by calculating RIN values as described previously [4]. Purified RNA was reverse transcribed using high capacity cDNA reverse transcription kit (Invitrogen) following the manufacturer protocol. Real time PCR amplifications were performed in CFX96 Real Time System (Biorad) with the following cycling parameters: an initial hot start of 95 °C for 3 min followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. In order to normalize qPCR reactions, *HPRT* (hypoxanthine phosphoribosyl-transferase) was included as reference gene [4]. The relative expression of genes were calculated based on the average Ct values across samples and computed the relative fold expression and significance using students t-test comparing control versus patient mean values.

3. Results

Our data showed that the relative normal expression of *MVP* and *BCRP* were significantly higher in both the pathologies with respect to the non-epileptic autopsy (H/F) controls. MTLE/H; *MVP*: 2.73 ± 0.178 ($p < 0.05$), *BCRP*: 18 ± 0.34 ($p < 0.01$), FCD/FL; *MVP*: 5.86 ± 0.087 ($p < 0.05$), *BCRP*: 2.54 ± 0.132 ($p < 0.01$), whereas *MRP1* and *UGT1A4* were unaltered, MTLE (n = 16), FCD (n = 12), H

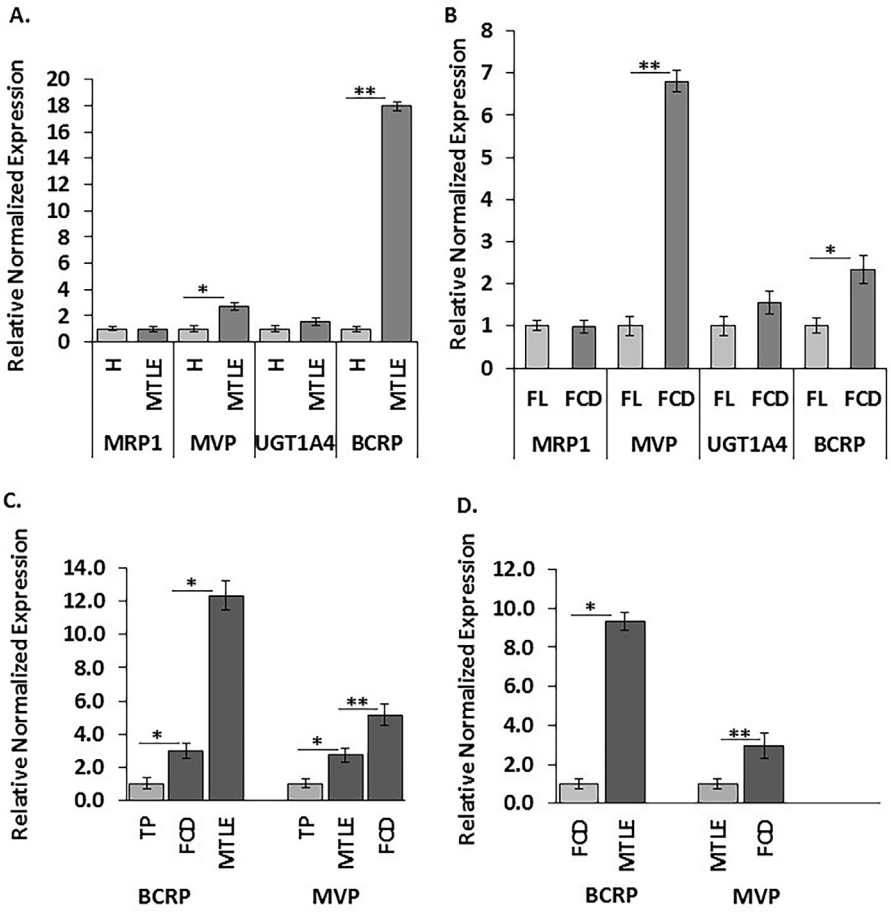

Fig. 1. Gene expression analysis using qPCR. A. mRNA levels of MRP1, MVP, BCRP and UGT1A4 in MTLE as compared to Hippocamapal autopsy control (H), B. mRNA levels of MRP1, MVP, BCRP and UGT1A4 in FCD as compared to frontal lobe autopsy (FL) as control, C. mRNA levels of BCRP and MVP in MTLE and FCD as compared tumour periphery (TP) controls, D. mRNA levels of MVP and BCRP in MTLE and FCD with respect to each other. MVP and BCRP mRNA shows significant upregulation (A–D) whereas MRP1 and UGT1A4 expression levels are unaltered as compared to controls in both MTLE and FCD patients (A, B). BCRP upregulation was significantly higher in MTLE as compared to FCD, whereas MVP upregulation was significantly higher in FCD as compared to MTLE (D). Mean increase in transcripts levels were statistically significant (*$P < 0.05$; **$P < 0.01$). Error bar is ±SD based on 16 MTS (M1–M16), 12 FCD (F1–F12), 6 tumor periphery control (C1–C6) and 4 autopsy control (A1–A4) patients, each done in triplicate.

(n = 4), FL (n = 4), TP (n = 6). To validate the upregulation observed in *MVP* and *BCRP*, qPCR was performed using tumour periphery control tissues. Relative normal expression of *MVP* and *BCRP* was significantly higher (MTLE/TP; *MVP*: 2.73 ± 0.43 ($p < 0.05$), *BCRP*: 12.34 ± 0.87 ($p < 0.05$), (FCD/TP; *MVP*: 5.16 ± 0.67 ($p < 0.05$), *BCRP*: 2.99 ± 0.45 ($p < 0.01$) in both the pathologies even with respect to the tumor periphery controls. Relative normal expression of *BCRP* was significantly higher in MTLE (9.34 ± 0.45; $p < 0.05$) with respect to FCD whereas relative expression of *MVP* was significantly higher (2.94 ± 0.65; $p < 0.01$) in FCD with respect to MTLE (Fig. 1).

4. Discussion

Till date there are numerous studies demonstrating overexpression of drug transporters such a P-gp and members of MRP family in epileptogenic brain tissue surgically resected from patients with DRE, however very few studies have focussed on other molecules involved in drug resistance such as MVP, BCRP and UGT1A4 [9–16]. Our data shows significant alterations in the levels of *MVP* and *BCRP* whereas *MRP1* and *UGT1A4* were unaltered in both MTLE and FCD. We report significantly higher upregulation of *BCRP* and *MVP* in MTLE and FCD respectively and propose that *BCRP* and *MVP* may have the potential to predict multidrug resistance phenotypes in these pathologies. With the current data it is difficult to predict the causes, functions or pathology specificities of these molecules and needs to be explored in future. Although *MRP1* expression is quite contradictory to existing literature as being the major transporter across the BBB, we cannot rule out the modulations in the levels of other significant transporters across the BBB like MDR1 [10–13]. As BCRP is more abundant than MRPs in the brain, upregulation of *BCRP* in the hippocampal tissues resected from MTLE might modulate the bioavailability of the AEDs [13]. Our finding that *MVP* levels are higher in samples obtained from patients with FCD (Type I and II) is in line with previous reports showing higher MVP upregulation in FCD cases, although these studies included patients with dual pathology [14]. MVP, by restricting compartmentalization of the drugs, might contribute significantly towards drug resistance in FCD. Whereas, BCRP by causing efflux of the drugs might be contributing to the drug resistance in MTLE [14,16]. These results suggest involvement of more than one molecule with different drug resistance mechanisms in a particular DRE pathology. BCRP and MVP may play a crucial role in the development of drug resistance in MTLE and FCD respectively and may serve as potential prognostic/diagnostic markers of DRE. As these molecules can be blocked by specific inhibitors, such inhibitors can therefore be used as adjunctive treatment for DRE. In this study, we are also reporting for the first time that tumour periphery controls show comparable results with the autopsy controls and thus propose that tissues resected from tumour margins can serve as good non-epileptic controls and should be included in future studies along with autopsy controls to overcome the disadvantages associated with either controls. In conclusion our study shows association of *BCRP* and *MVP* in MTLE and FCD and warrants further investigations in a larger cohort of patients. Studies on larger cohort will aid in more specific clinical correlation to look for their potential as diagnostic/prognostic markers of MTLE and FCD which might further lead to personalised and targeted drug resistance therapies in future.

Conflict of interests

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.seizure.2017.02.014.

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