Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy

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Summary

Epilepsy is resistant to drug treatment in about one-third of cases, but the mechanisms underlying this drug resistance are not understood. In cancer, drug resistance has been studied extensively. Amongst the various resistance mechanisms, overexpression of drug resistance proteins, such as multi-drug resistance gene-1 P-glycoprotein (MDR1) and multidrug resistance-associated protein 1 (MRP1), has been shown to correlate with cellular resistance to anticancer drugs. Previous studies in human epilepsy have shown that MDR1 and MRP1 may also be overexpressed in brain tissue from patients with refractory epilepsy; expression has been shown in glia and neurones, which do not normally express these proteins. We examined expression of MDR1 and MRP1 in refractory epilepsy from three common causes, dysembryoplastic neuroepithelial tumours (DNTs; eight cases), focal cortical dysplasia (FCD; 14 cases) and hippocampal sclerosis (HS; eight cases). Expression was studied immunohistochemically in lesional tissue from therapeutic resections and compared with expression in histologically normal adjacent tissue. With the most sensitive antibodies, in all eight DNT cases, reactive astrocytes within tumour nodules expressed MDR1 and MRP1. In five of eight HS cases, reactive astrocytes within the gliotic hippocampus expressed MDR1 and MRP1. Of 14 cases of FCD, MDR1 and MRP1 expression was noted in reactive astrocytes in all cases. In five FCD cases, MRP1 expression was also noted in dysplastic neurones. In FCD and DNTs, accentuation of reactivity was noted around lesional vessels. Immunoreactivity was always more frequent and intense in lesional reactive astrocytes than in glial fibrillary acidic protein-positive reactive astrocytes in adjacent histologically normal tissue. MDR1 is able to transport some antiepileptic drugs (AEDs), and MRP1 may also do so. The overexpression of these drug resistance proteins in tissue from patients with refractory epilepsy suggests one possible mechanism for drug resistance in patients with these pathologies. We propose that overexpressed resistance proteins lower the interstitial concentration of AEDs in the vicinity of the epileptogenic pathology and thereby render the epilepsy caused by these pathologies resistant to treatment with AEDs.

Introduction

Epilepsy is resistant to drug treatment in about one-third of cases overall (Sander, 1993). The proportion of cases resistant to drug treatment varies with the specific cause or syndromic diagnosis. Thus, notwithstanding selection bias, a higher proportion of subjects with epilepsy due to hippocampal sclerosis (HS), malformations of cortical development and dysembryoplastic neuroepithelial tumours (DNTs) are likely to have refractory epilepsy (Daumas-Duport, 1993; Guerrini *et al.*, 1996; Semah *et al.*, 1998). The basis of resistance to drug treatment is not known, but is likely to be multifactorial.

Most patients resistant to drug treatment do not become seizure free with any of a broad range of antiepileptic drugs (AEDs). AEDs have a variety of postulated antiepileptic actions, but tend to be physically similar; for example, most AEDs are lipophilic (Levy *et al.*, 1995). These phenomena suggest the involvement of non-specific mechanisms of resistance. Drug resistance is also a major therapeutic problem in cancer. Overactivity of a range of drug resistance pathways has been shown in individual drug-resistant neoplastic cells (Hipfner *et al.*, 1999*a*; Tan *et al.*, 2000). Amongst the best understood mediators of drug resistance are multidrug resistance gene-1 P-glycoprotein (MDR1; also known as ABCB1) and multidrug resistance-associated protein 1 (MRP1; also known as ABCC1) (see reviews by Ling, 1997; Cole and Deeley, 1998; Borst *et al.*, 2000). Both MDR1 and MRP1 are members of the ABC (ATP-binding cassette) transporter protein superfamily. Both proteins reduce compartmental, cytoplasmic or organelle drug accumulation as a result of transmembrane drug transport, either by extracellular drug export or by intracellular vesicular sequestration (Ling, 1997; Van Luyn *et al.*, 1998; Hipfner *et al.*, 1999*a*; Wijnholds *et al.*, 2000). Thus, MDR1 or MRP1 overexpression causes constitutive or acquired resistance to anticancer drugs, and worsens prognosis (Sonneveld, 2000; Tan *et al.*, 2000). Overcoming drug resistance protein activity may influence treatment and prognosis in some cancers (Tan *et al.*, 2000).

The normal human brain is protected by both MDR1 and MRP1, which contribute to the blood–brain and blood–CSF barriers (Schinkel, 1999; Wijnholds *et al.*, 2000). In normal human brain, MDR1 is found only in the vicinity of blood vessels, most probably on endothelial cell membranes, whilst MRP1 is found only in the choroid plexus epithelium (Seetharaman *et al.*, 1998; Rao *et al.*, 1999). Drawing on parallels between drug resistance in cancer and epilepsy, a number of preliminary reports have documented brain overexpression of MDR1 or MRP1 in refractory epilepsy. In refractory temporal lobe epilepsy due to a range of causes, Tishler *et al.* (1995) demonstrated MDR1 overexpression histologically in glia in resected tissue. By constructing cell lines overexpressing MDR1 *in vitro,* they showed that MDR1 can transport phenytoin, indicating that its overexpression is a plausible cause of drug resistance. Lazarowski *et al.* (1999) showed in an uncontrolled study that there was MDR1 overexpression in resected epileptogenic tissue from a case of tuberous sclerosis. We found constitutive MDR1 overexpression in malformations of cortical development, with the histological demonstration of MDR1 in glia in malformed areas in post-mortem brain tissue from individuals who had never suffered seizures or had AED treatment (Sisodiya *et al.*, 1999). We have recently also shown overexpression of MRP1 in dysplastic neurones, glia and around intracerebral blood vessels in four surgical resection specimens containing focal cortical dysplasia (FCD), an important malformation of cortical development causing refractory epilepsy (Sisodiya *et al.*, 2001).

As an essential step in the further analysis of the possible contribution of drug resistance proteins MDR1 and MRP1 to drug resistance in epilepsy, we undertook a histological study of their expression in human brain tissue from subjects with three common causes of refractory epilepsy: FCD, DNTs and HS.

Methods

Tissue

We studied formalin-fixed paraffin-embedded human brain tissue from neuropathological archives. The study was approved by the Joint Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery. All case tissue was from therapeutic surgical resections for refractory epilepsy and was surplus to diagnostic requirements. All samples were anonymized. Routine staining (haematoxylin and eosin or cresyl violet) was performed to confirm the histological diagnosis and to provide anatomical detail.

Sections with epileptogenic pathology had ideal fixation conditions, suffering no significant pre-resection hypoxia, being immersed in formalin immediately and embedded within 1 week. All cases had been exposed to multiple (at least three) AEDs. Control tissue was of two types: (i) histologically normal adjacent brain tissue from the same focal resection specimen; this tissue allowed control for age, sex, region of brain, exposure to AEDs, direct and indirect effects of seizures and tissue preservation; and (ii) positive control tissue was either normal human liver, kidney or choroid plexus or, as a positive disease control, breast carcinoma. We previously have demonstrated effectively that duration of fixation *per se* is unlikely to reduce MDR1 or MRP1 expression (Sisodiya *et al.*, 1999, 2001).

Eight cases of DNT were examined, all of which were large excisions (Cases 1–8). Each case showed typical features of DNT, with intracortical nodules of glial, neuronal and oligodendrocyte-like cells, and with separate glioneuronal elements. In all cases, there was adjacent normal appearing cortex. Eight cases of HS were studied (Cases 9–16). All showed the typical pattern of cell loss involving CA4 and CA1 with sparing of the CA2 sector in all but one severely affected case. Fourteen cases of FCD were examined. In six cases (Cases 17, 18 and 22–25), the specimens were large resections with adjacent normal cortical tissue for comparison. All cases were characterized histologically by the presence of large dysplastic neurones, highlighted with silver and neurofilament stains, disordered laminar architecture and variable numbers of balloon cell glia, often located in the deeper regions of the cortex and the underlying white matter.

Antibodies

For detection of MRP1, monoclonal antibodies MRPr1 and MRPm6 (1 : 100 dilution; Alexis Corporation, Nottingham,
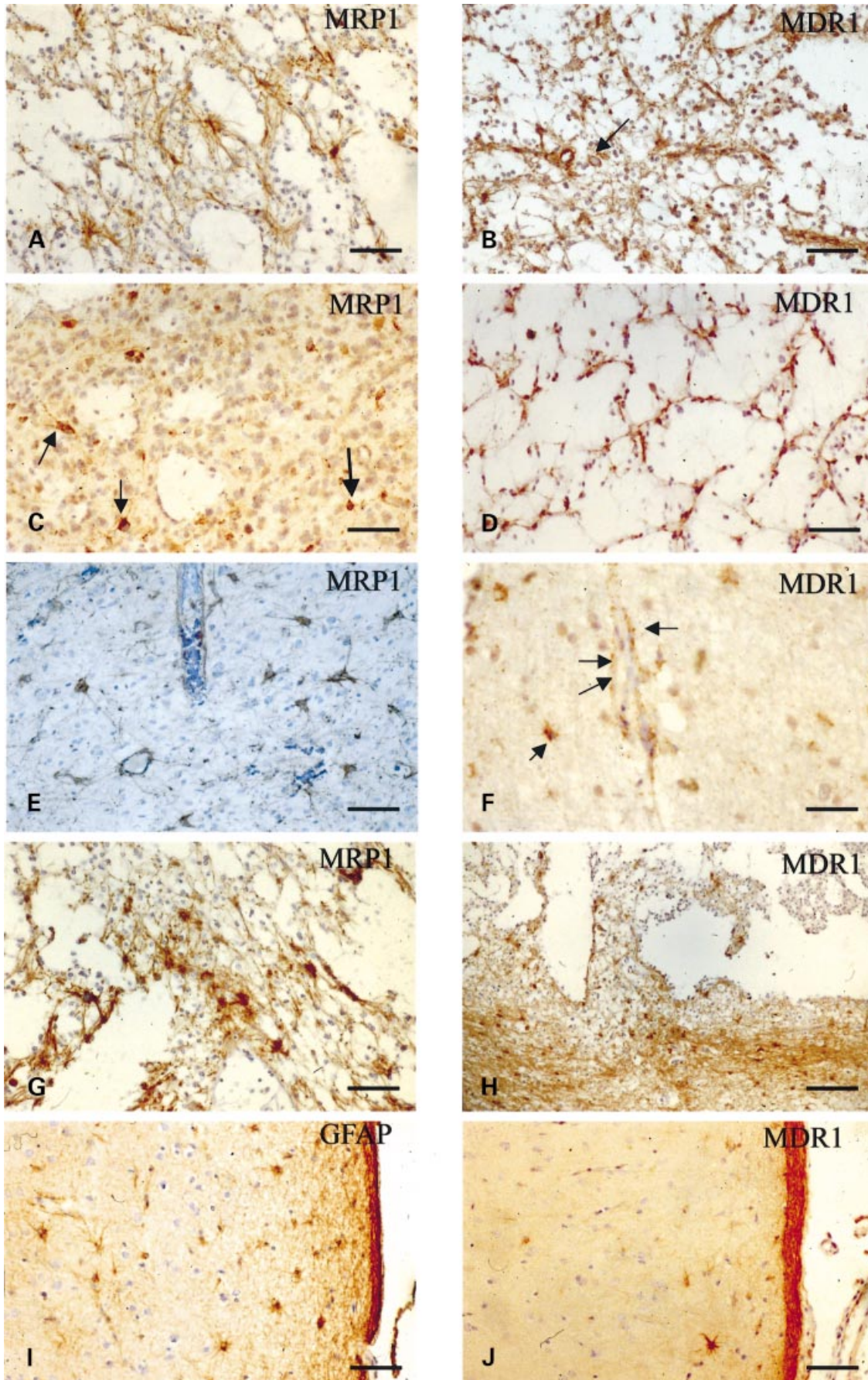
UK) were used (Flens *et al*., 1994). The antibodies have been well characterized, recognize separate internal epitopes of the MRP1 molecule (Hipfner *et al*., 1998, 1999*b*) and are believed to be specific for MRP1, with no reported cross-reactivity (Scheffer *et al*., 2000). Paraffin-embedded sections of human kidney and choroid plexus epithelium, known to express MRP1 (Flens *et al*., 1996), were used as positive controls. To detect MDR1 expression, the well-characterized monoclonal antibodies C494 (1 : 250 dilution; Alexis Corporation) and C219 (1 : 100 dilution; Calbiochem-Novabiochem Co., San Diego, Calif., USA) were used; they recognize separate internal epitopes of the MDR1 molecule (Beck *et al*., 1996; van Den Elsen *et al*., 1999; Scheffer *et al*., 2000). C494 is known to cross-react with pyruvate carboxylase (Rao *et al*., 1994), whilst C219 cross-reacts with c-erbB2 in cancer cells, though with significantly less affinity (Liu *et al*., 1997; van Den Elsen *et al*., 1999). Normal liver was used as positive control for MDR1 antibodies, whilst, within experimental sections, labelling of capillary endothelium acted as a positive internal control.

Immunohistochemistry

Sections of 10 μm were cut and mounted on APTES (3-aminopropyltriethoxysilane; Sigma, Poole, UK) coated microscope slides. Sections were dried at 37°C overnight, then dewaxed in xylene for 15 min prior to rehydration in graded alcohols (100, 95 and 70%). Endogenous peroxidase activity was inhibited by incubation in 6% (v/v) H_2O_2 in methanol for 30 min, followed by washing in tap water. Antigen retrieval was performed by boiling sections in 0.01 M citrate buffer (pH 6.0) for 5 min. Non-specific protein binding was blocked with 20% normal goat serum in Tris-buffered saline (TBS; 50 mM Tris–HCl/150 mM NaCl, pH 7.6) for 30 min at room temperature. After incubation overnight at 4°C in the diluted primary antibody, sections were washed twice for 15 min in TBS. Bound antibodies were detected using biotinylated anti-species-specific immunoglobulins (anti-mouse IgG, 1 : 100 dilution for C494, C219 and MRPm6; anti-rat IgG, 1 : 100 dilution for MRPr1). Sections were washed twice in TBS for 15 min, and then developed

using avidin–peroxidase (1 : 400 dilution in 0.125 M TBS). The immunoreaction was developed with 0.05% (w/v) 3,3-diaminobenzidine activated with 0.01% (v/v) H_2O_2. After washing in running tap water for 5 min, sections were counterstained with haematoxylin, and mounted with DPX. Glial fibrillary acidic protein (GFAP) immunohistochemistry was performed according to routine protocols.

All sections were reviewed independently by four observers, including three neuropathologists. Blinding was not possible for neuropathology. For each antibody run, experimental sections were assessed only if positive and negative (obtained with omission of primary) controls reacted as predicted. No labelling was noted with omission of the primary antibodies. The presence and degree of gliosis were determined from GFAP-labelled sections.

Results

Dysembryoplastic neuroepithelial tumours

Positive immunostaining, to variable degrees, was noted for MRP1 and for MDR1 within DNT nodules in all cases with antibodies MRPr1 and C494, respectively. Positive immunostaining was noted in four out of eight cases with C219, and four out of seven cases with MRPm6. The pattern of positive immunostaining in labelled cases was similar with both the antibodies for each molecule under study. In four cases, C219 did not label capillary endothelium, the internal positive control.

Immunopositive cells had the morphology of reactive astrocytes (Fig. 1A–G). The oligodendrocyte-like cells and mature neuronal element in general did not label with these antibodies (Fig. 1), although in one case only there was peripheral labelling for MDR1 around large neurones (Fig. 1B). Striking positivity of the astrocyte foot processes on the fine anastomozing capillary networks within the DNT was noted with MRPr1 antibody (Fig. 1E), whereas there were meshworks of perivascular fibres labelled for MDR1 within the nodules with both anti-MDR1 antibodies (Fig. 1B and D). Within individual cases, there was heterogeneity in the number of positive cells in different regions of the tumour,

Fig. 1 Dysembryoplastic neuroepithelial tumour immunostaining patterns with antibodies against MRP1 (**A**, **C**, **E** and **G**) and MDR1 (**B**, **D**, **F**, **H** and **J**). (**A**) A cellular nodule within a DNT composed mainly of small oligodendrocyte-like cells with a microcystic pattern. Staining for MRP1 (with MRPr1) highlights distinct populations of cells with astrocytic morphology and prominent cellular processes. (**B**) Labelling of the same DNT for MDR1 (with C494) shows positivity of a proportion of small cells and processes, some of which appear to surround capillaries. In addition, peripheral labelling of occasional cells with the morphology of larger neurones within the DNT was seen (arrow). In another DNT (**C**), labelling for MRP1 (with MRPm6) showed that some of the small cells within a cellular nodule were immunopositive (arrows), some having cellular processes, and (**D**) labelling for MDR1 (with C219) showed similar focal positivity in a proportion of small cells and processes. (**E**) Immunopositivity for MRP1 with MRPr1 was also seen on astrocyte-like cells with foot processes extending onto capillaries. (**F**) Immunostaining for MDR1 (with C219) also showed punctate positivity around a capillary (long arrows) and labelling of occasional small astrocyte-like cells at the margins of a nodule (short arrow). (**G**) Greater numbers of immunopositive astrocyte-like cells were seen in some DNTs with MRPr1. In some cases (**H**) with C494 for MDR1, accentuation of labelled cells and condensation of processes was seen in the periphery of a nodule, which was also observed with MRP1 antibodies. The cortex adjacent to the DNT typically showed a moderate subpial and cortical gliosis with reactive astrocytes on GFAP (**I**), but relatively less staining was observed in the same regions for MRP1 (not illustrated) and MDR1 (**J**). Magnification: in **H**, bar = 150 μm; all others, bar = 60 μm.
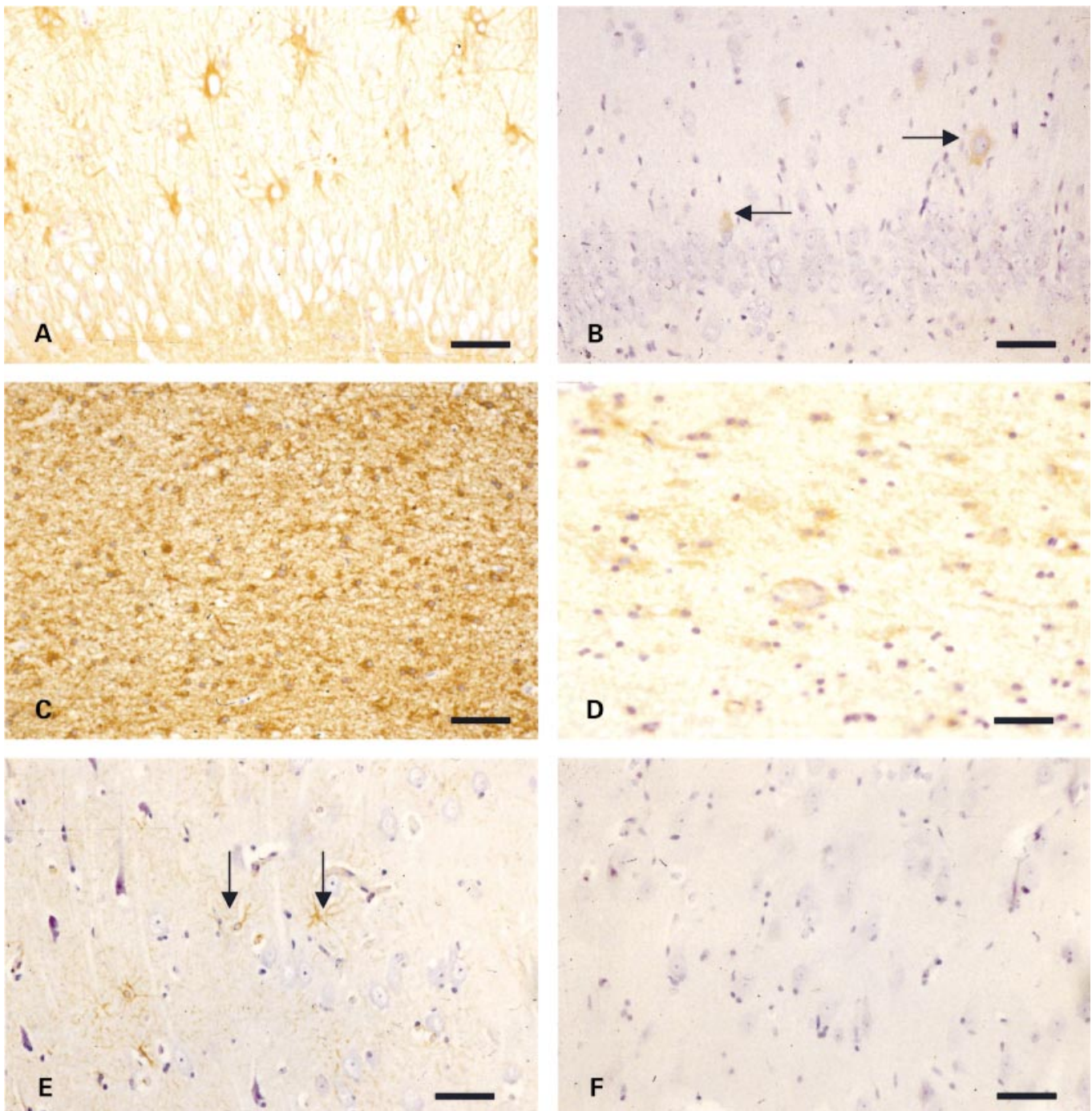
Fig. 2 Hippocampal sclerosis. (**A**) Dentate gyrus granule cell and molecular layers showing prominent reactive astrocytes with GFAP immunohistochemistry and (**B**) astrocytic immunopositivity (arrowed) for MRP1 with MRPr1 antibody. (**C**) CA1 sector showing neuronal loss and dense GFAP-immunopositive fibre meshworks and (**D**) a proportion of glial cells also showing immunopositivity for MDR1 with C494 antibody. Reactive astrocytes were demonstrated in the adjacent subiculum of these cases with GFAP immunostaining (**E**; arrowed) but no immunolabelling was seen for either MRP1 (**F**) or MDR1 (not illustrated) in this same area. Magnification: in all, bars = 51 μm.

with some nodules showing fewer labelled cells than others. There were perinodular meshworks of MDR1-positive fibres in some cases (e.g. Case 6; Fig. 1H). Whilst GFAP labelling revealed reactive astrocytes in the underlying white matter in all cases, in only one case was there marked labelling for

MRP1 of subnodular white matter astrocytes, in a perivascular distribution.

Immunoreactivity with antibodies for both proteins in adjacent normal cortex in all cases was generally restricted to subpial meshworks of labelled processes (corresponding to

Chaslin's gliosis) and layer I astrocytic cells, despite evidence of moderate cortical gliosis with numerous reactive astrocytes demonstrated by GFAP immunostaining (Fig. 1I, GFAP; Fig. 1J, MDR1). This immunoreactivity was often qualitatively less intense than that seen in the DNT itself.

Hippocampal sclerosis
GFAP immunostaining confirmed dense fibrillary gliosis in CA1 and CA4 in all cases, with prominent reactive astrocytes noted in the dentate gyrus molecular layer in one case (Case 16; Fig. 2A). MRP1 immunopositivity with MRPr1 antibody was seen in five cases (Cases 10, 12, 13, 15 and 16), and localized to cells with the morphology of reactive astrocytes (Fig. 2B), although qualitatively the immunoreaction appeared weaker than that observed in the DNT. MRPm6 antibody did not label any of these cases. MDR1 immunostaining was confined to astrocytic cells in the hippocampus and was observed in five cases (Cases 10, 11, 14, 15 and 16) with C494 antibody, but with a subjectively less intense reaction than noted in DNT cases (Fig. 2C and D). No immunostaining of astrocytes or capillary endothelium was noted in five cases studied with C219 antibody. Immunostaining was not present in the adjacent subiculum in any of the cases with either set of antibodies, despite the presence of GFAP-positive reactive astrocytes (Fig. 2E and F).

Focal cortical dysplasia
GFAP immunostaining showed variable positivity in a proportion of the balloon cells and highlighted a variable degree of reactive astrocytosis. In three cases (Cases 17, 21 and 22), there were dense meshworks of GFAP-positive fibres in the region of dysplasia.

Staining for MRP1 was present in dysplastic neurones in five cases (Fig. 3A and B), in foot processes around vessels (Fig. 3C) and in balloon glia in two cases (not illustrated). Prominent and distinctive MRP1-positive processes were seen enveloping dysplastic neurones (Fig. 3D) and accentuation of labelling was seen in foot processes around vessels when compared with the GFAP. Labelling of reactive astrocytes for MRP1 and MDR1, seen in all cases with MRPr1 and C494 (Fig. 3E and F) antibodies, respectively, was distinctly more pronounced in the area of dysplasia compared with the adjacent cortex. In Case 17, staining for MRP1 specifically highlighted discrete islands of dysplastic balloon cells in the white matter (Fig. 3G and H), whereas the GFAP staining in this case showed a more widespread white matter gliosis, albeit with accentuation in these islands. Immunostaining for MDR1 was similar but less intense in three cases (Cases 17, 20 and 22). The adjacent normal cortex, where available for study, showed markedly fewer astrocytes immunostained for MDR1 and MRP1, even though gliosis was demonstrated with GFAP in these cases (Fig. 3I, GFAP; Fig. 3J, MRP1). No normal neurones were labelled

with either antibody. Glial immunolabelling was similar in four out of six cases with MRPm6 antibody for MRP1, but C219 antibody for MDR1 protein did not label astrocytes or normal capillary endothelium in any of these six cases.

Discussion
In conditions associated with resistance to drug treatment, there must be mechanisms that make some cases more resistant to treatment than others. Detection of potential candidates mediating resistance is the first step in the management of resistance. In pathologies commonly causing refractory epilepsy, we have shown the presence of known mediators of drug resistance, MDR1 and MRP1, in lesional glia and, for FCD, in a proportion of lesional dysplastic neurones. Normal human glia do not have detectable expression of either protein under normal conditions using immunohistochemistry (Tishler *et al.*, 1995; Seetharaman *et al.*, 1998) and, as far as we can determine, neither do normal neurones. Whether expression occurs below the threshold for detection with immunohistochemistry is not known: 'overexpression' rather than a novel cellular phenotype is a more conservative interpretation. Thus, we consider that detection of MDR1 and MRP1 in glia in HS, DNT and FCD and of MRP1 in some dysplastic neurones in FCD represents their overexpression in pathologies that cause refractory epilepsy. These findings extend previous reports of overexpression of MDR1 and MRP1 in epileptogenic brain tissue (Tishler *et al.*, 1995; Lazarowski *et al.*, 1999; Sisodiya *et al.*, 1999, 2001). In the case of dysplastic neurones in FCD, however, the MRP1-positive phenotype may be part of an overall cytological abnormality, as these dysplastic neurones are known to express a number of unusual phenotypes (Hamberger *et al.*, 1993; Duong *et al.*, 1994; Wolf *et al.*, 1995; Yamanouchi *et al.*, 1996; Garbelli *et al.*, 1999).

Immunohistochemical study of MDR1, in particular, is complicated by the cross-reactivity of some anti-MDR1 antibodies (Beck *et al.*, 1996). For this reason, we used two separate antibodies. The antibodies used, C494 and C219, are both known to cross-react: C494 with a ubiquitous mitochondrial enzyme, pyruvate carboxylase (Rao *et al.*, 1994), and C219 with a different protein in cancer cells (c-erbB2; Liu *et al.*, 1997). However, immunohistochemistry is complicated further by variability in immunostaining with different antibodies under identical test conditions, especially in fixed pathological tissue as opposed to control tissues (Beck *et al.*, 1996), and C219 is less sensitive than C494 (Lacueva *et al.*, 1998). We believe that immunostaining with C494 and C219 antibodies is most likely to represent labelling of MDR1 for the following reasons. (i) In pathology with the strongest labelling, DNTs, the pattern of labelling was similar with both antibodies when the antibody of second choice (C219) labelled sections. In the DNT, HS and FCD cases in which C219 did not label reactive astrocytes, it also did not label the internal control (capillary endothelium), reflecting the known lower sensitivity of C219 antibody in paraffin
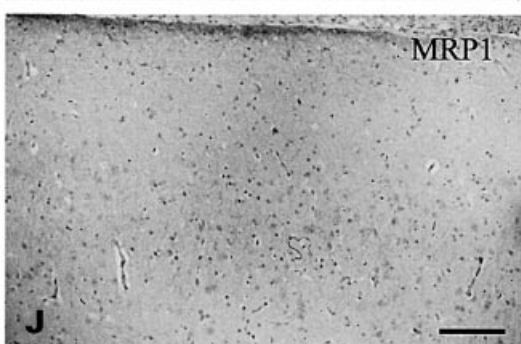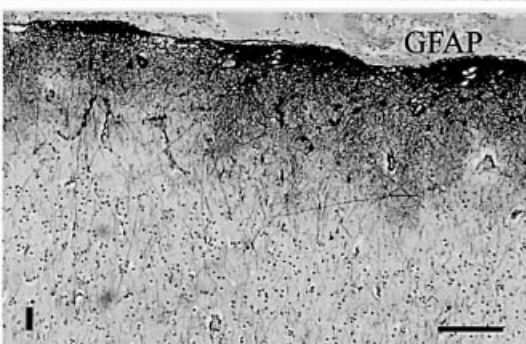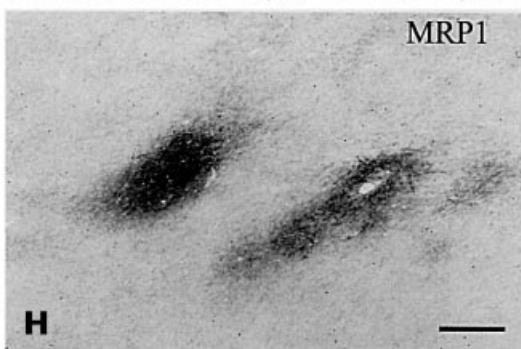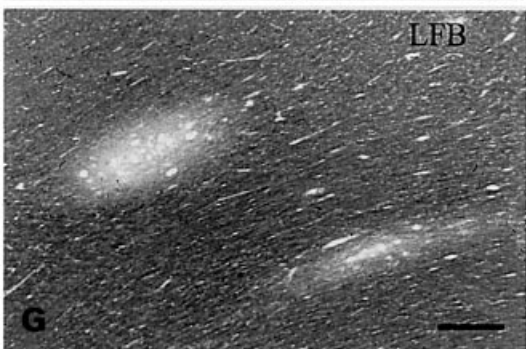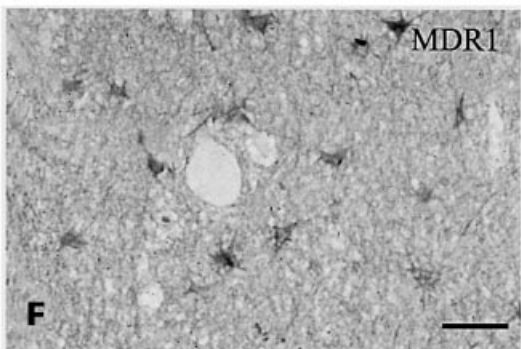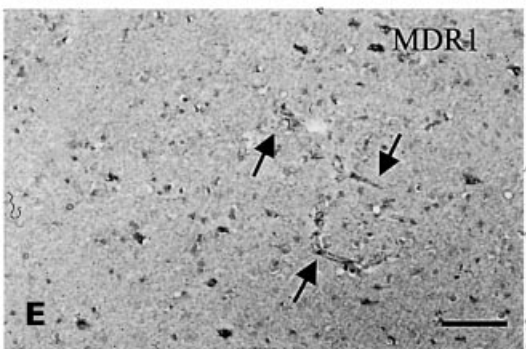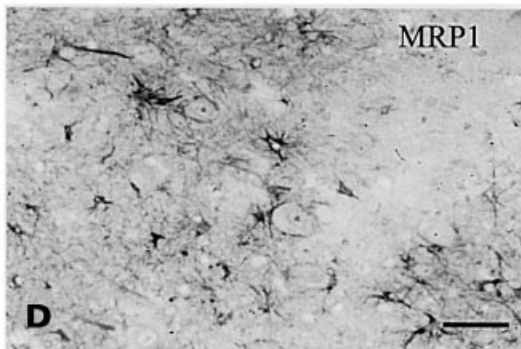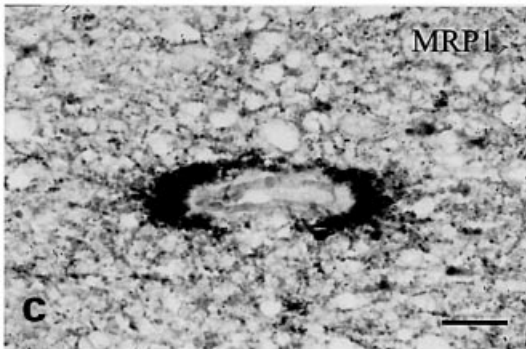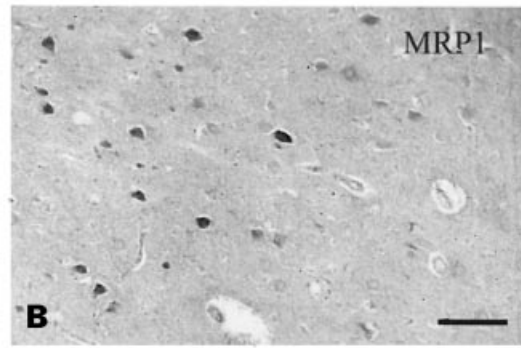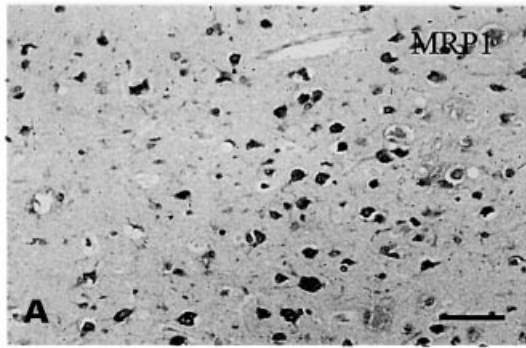
sections (Lacueva *et al*., 1998), possibly in combination with lower levels of overexpression in HS and FCD compared with DNTs as judged qualitatively by intensity of immunolabelling with C494. (ii) Although reactive astrocytes are plentiful lesionally and perilesionally, the great majority of immunostaining with C494 and C219 occurs only lesionally, and not throughout the more widespread areas of gliosis. (iii) At a microscopic level, intense immunostaining with C494 and C219 is noted in thin astrocytic processes and astrocytic endfeet, where mitochondria are unlikely to be found (Peters *et al*., 1991); for the MRP1 antibodies, no cross-reactivity has been reported with either (Hipfner *et al*., 1999*b*; Scheffer *et al*., 2000). We were able to show similar immunostaining patterns for both MRP1 antibodies in DNT and FCD cases, confirming that the protein detected is most likely to have been MRP1. The weaker immunolabelling with MRPm6 in fixed tissue has been reported previously (Scheffer *et al*., 2000), and may reflect differential effects of fixation on the relevant (separate) epitopes. MRP1 and MDR1 have only 15% amino acid identity (Cole and Deeley, 1998); the antibodies we have chosen do not cross-react with these two proteins (Flens *et al*., 1994; Hipfner *et al*., 1998; Scheffer *et al*., 2000). Therefore, the proteins detected in this study are likely to be MDR1 and MRP1, although we cannot completely exclude other cross-reacting antigens.

Comparison with brain tissue from subjects with non-refractory epilepsy might be thought to be ideal. Such tissue is not available for study in routine practice, as such subjects do not, as a rule, undergo surgical treatment for their epilepsy. Conversely, resected histologically normal brain tissue adjacent to epileptogenic tissue was of the same age, sex and brain region, and had been exposed to the same seizure effects and drugs, and was therefore ideal disease control tissue. Immunohistochemistry of adjacent normal tissue in cases from each of the pathologies we have studied demonstrates reduced immunoreactivity for MDR1 and MRP1 irrespective of the extent of gliosis. Immunohistochemically detectable overexpression therefore appears limited to the extent of observable epileptogenic pathology in these resection specimens. We previously have shown constitutive overexpression of MDR1 in other malformations (Sisodiya *et al*., 1999). Overexpression of drug resistance proteins in HS, FCD and DNTs, whether constitutive or induced, is thus likely to be an intrinsic property of these pathologies. In normal appearing

brain, we have shown that seizures, AEDs and other effects of epilepsy need not cause overexpression, even though drugs including phenobarbitone (Schuetz *et al*., 1996; Chan *et al*., 1997), cellular stress and hypoxia (Gomi *et al*., 1997; Vilaboa *et al*., 2000) are known to cause overexpression in some cell lines *in vitro*.

The distribution of immunostaining is intriguing. As illustrated in Figs 1B, E and F, and 3A–E, immunoreaction appears most marked around vessels and dysplastic neurones. MDR1 contributes to the blood–brain barrier, whilst MRP1 has a role in regulating CSF constitution at the choroid plexus (Hipfner *et al*., 1999*a*; Rao *et al*., 1999; Schinkel, 1999; Wijnholds *et al*., 2000). The normal endothelial blood–brain barrier may be disrupted in seizures (Yaffe *et al*., 1995), and glial overexpression may represent a 'second barrier'. The cytoplasmic appearance of immunostaining may obscure any underlying membranous labelling. Such localization of MDR1 and MRP1 would be the most likely for drug export from interstitial CSF, but intracellular activity of both proteins has also been demonstrated to be important (Van Luyn *et al*., 1998; Merlin *et al*., 2000; Meschini *et al*., 2000; Tan *et al*., 2000).

From our study of fixed material, we cannot determine whether the proteins detected are functionally active. Absence or inhibition of MDR1 and MRP1 can lead to excessive CSF penetration of a range of molecules (Schinkel, 1999; Wijnholds *et al*., 2000), whilst overexpression is associated with resistance to anticancer treatment in some neurological malignancies (Abe *et al*., 1998). MDR1 can transport phenytoin (Tishler *et al*., 1995) and phenobarbitone (Schuetz *et al*., 1996) and is known to be able to transport other planar lipophilic molecular structures: most current AEDs are planar and lipophilic (Levy *et al*., 1995). MRP1 may be able to transport other AEDs, and is known to transport drug epoxides and glucuronides, into which the conjugates carbamazepine and lamotrigine are metabolized (Levy *et al*., 1995). It is possible, therefore, that MDR1 and MRP1 overexpressed in the pattern observed in our cases might lower local interstitial AED concentration and thereby reduce their antiepileptic effects. We have suggested previously that neuronal MRP1 expression might also modulate other effects of AEDs (Sisodiya *et al*., 2001).

This is an observational study, and we cannot in this context explore the possible functional consequences of

Fig. 3 Focal cortical dysplasia. Dysplastic neurones showed immunopositivity with anti-MRP1 antibodies MRPm6 (**A**) and MRPr1 (**B**)* in a proportion of cases. (**C**) Prominent labelling for MRP1 with MRPr1 was seen in perivascular processes in the vicinity of the dysplasia. (**D**) MRPr1 also labelled cells with the morphology of reactive astrocytes in the area of cortical dysplasia, with processes appearing to envelop dysplastic neurones, the neurones themselves being immunonegative in this case. Immunopositivity with C494 antibody for MDR1 was seen around small capillaries (**E**; arrows) and small glial cells in the vicinity of dysplasia (**E** and **F**), but did not label balloon cell glia (**F**). Islands of dysplastic balloon cell glia in the underlying white matter, as seen with the Luxol fast blue/Nissl preparation (**G**), showed specific labelling for MRP1 (**H**), whereas labeling with GFAP showed a more diffuse immunopositivity reflecting the severe white matter gliosis (not shown). The normal cortex adjacent to the cortical dysplasia, where available, showed a variable superficial astrocytic gliosis (**I**), whereas little immunostaining was demonstrated for MDR1 or MRP1 (**J**) from corresponding regions in adjacent sections. Magnifications: **A**, **B**, **E**, **I** and **J**, bar = 150 μm; **C**, bar = 36 μm; **D** and **F**, bar = 60 μm; **G** and **H**, bar = 570 μm. *Fig. 3B is reproduced from Sisodiya *et al*. (2001), with permission from the copyright holders, The Lancet Ltd.

overexpression. The results suggest that investigation of the AED and AED conjugate transport capacity of MDR1 and MRP1 might be worthwhile. If MDR1 and MRP1 are able to transport or sequester AEDs, new options might eventually be considered for the adjunctive treatment of refractory epilepsy, although functional polymorphisms (Hoffmeyer *et al.*, 2000)—not necessarily detectable immunohistochemically—and the adverse effects of inhibition, would need careful consideration.

Acknowledgements

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