Significance of the expression of MRP1 and MRP2 in peripheral blood mononuclear cells of children with intractable epilepsy

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Abstract. Intactable epilepsy (IE) is relatively common in pediatric epilepsy. The resistance mechanism of IE has been previously investigated. Multidrug-resistant associated protein 1 (MRP1) and MRP2 are associated with drug transport. The aim of the present study was to investigate the expression of MRP1 and MRP2 in peripheral blood mononuclear cells of children with IE. Fifty outpatient or inpatient children were included in the study as the experimental group. Additionally, 50 children with epilepsy controlled by anti-epileptic drugs (AEDs) and 50 healthy children without epilepsy, who served as the control group, were included in the present study. Expression of MRP1 and MRP2 in the peripheral blood mononuclear cells of children in all the groups was detected using RT-PCR and western blot analysis. The results showed that the relative expression of MRP1 and MRP2 mRNA in the peripheral blood mononuclear cells of children with IE (MRP1, 0.795±0.042; MRP2, 0.804±0.023) was higher than that in epilepsy controlled by AEDs (MRP1, 0.682±0.030; MRP2, 0.675±0.021) and healthy children without epilepsy (MRP1, 0.665±0.031; MRP2, 0.654±0.029) (P<0.01). The mean relative expression of MRP1 and MRP2 protein in the peripheral blood mononuclear cells of children with IE (MRP1, 2.027±0.034; MRP2, 1.902±0.021) was higher than that in children with epilepsy controlled by AEDs (MRP1, 1.131±0.042; MRP2, 1.086±0.027) and healthy children without epilepsy (MRP1, 1.093±0.023; MRP2, 1.045±0.018) (P<0.01). The difference in the MRP1 and MRP2 mRNA and protein expression between the children with epilepsy controlled by AEDs and healthy children without epilepsy was not statistically significant (P>0.05). In conclusion, a higher expression of MRP1 and MRP2 in the peripheral blood mononuclear cells of children with IE may be relevant to the drug-resistant mechanism of IE.

Introduction

Intractable epilepsy (IE) accounts for 30-40% of the pediatric epilepsy and poses a challenge in pediatric neurology (1-2). In recent years, most studies investigating the resistance mechanism of IE have focused on cell death and reconstruction, overexpression of multidrug transporter, and changes of intracephalic drug targets (3,4). Previous studies focused on the association between multidrug transporter and epilepsy tolerance (5,6,15). The multidrug-resistant associated protein (MRP) is an important multidrug transporter, comprising nine MRPs (MRP1-MRP9), which are associated with drug transport. Among these, MRP1 and MRP2 may be related to the drug-resistant mechanisms of IE (7). MRP1 and MRP2 are distributed in the kidneys, liver, lungs, testes and peripheral blood mononuclear cells. They are also expressed in the luminal membrane on the basal side of endothelial cells or the luminal membrane of capillary endothelial cells in the choroid plexus, whose function is to restrict the entry of specific substances into the cerebrospinal fluid (8,9). The aim of the present study was to examine the expression of MRP1 and MRP2 protein in the peripheral blood mononuclear cells of children with IE using RT-PCR and western blot analysis. A higher relative expression of MRP1 and MRP2 mRNA and protein was identified in children with IE compared to the control groups. This higher expression indicates that IE may be relevant to the drug-resistant mechanism of IE.

Materials and methods

Subjects. For this study, 100 children with epilepsy from the outpatient or inpatient clinic of Xuzhou Children’s Hospital (Jiangsu, China) were enrolled between November 2010 and October 2013. The patients were into two groups. The IE group comprised 50 cases, all of which conformed to the criteria formulated by the International League Against Epilepsy (10). The criteria for enrollment were: exact clinical diagnosis, the application of two types of appropriate and tolerable anti-epileptic drugs failed to completely avoid epileptic seizure following adequate duration of treatment (duration-free from any epileptic seizure was considered as ≥3 times the longest interictal period prior to treatment or 12 months) and adequate doses of monotherapy or combinatorial treatment, with other nervous system disease history being excluded. Since the longest interictal periods in the enrolled patients was <3 months,
Table I. Expression of mRNA of MRP1 and MRP2 in peripheral blood mononuclear cells of children with intractable epilepsy (mean ± SD).

| Group                              | Cases | MRP1        | MRP2        |
|------------------------------------|-------|-------------|-------------|
| Group of healthy children without epilepsy | 50    | 0.665±0.031 | 0.654±0.029 |
| Group of children with epilepsy controlled by anti-epileptic drugs | 50    | 0.682±0.030<sup>a</sup> | 0.675±0.021<sup>a</sup> |
| Group of intractable epilepsy      | 50    | 0.795±0.042<sup>b</sup> | 0.804±0.023<sup>b</sup> |

F-value 121.364 292.194
P-value <0.001 <0.001

<sup>a</sup>P<0.001 when the group of children with intractable epilepsy was compared to the group of children with epilepsy controlled by anti-epileptic drugs and the group of healthy children without epilepsy. <sup>b</sup>P>0.05 when the group of children with epilepsy controlled by anti-epileptic drugs was compared to the group of healthy children without epilepsy. MRP, multidrug-resistant associated protein.

Analysis of the relative expression amount of mRNA of MRP1 and MRP2

To conduct the reverse transcription reaction, total RNA was extracted from the peripheral blood mononuclear cells using the Total RNA Extraction Kit (Takara, Dalian, China) to synthesize cDNA. The total reaction volume was 20 µl, consisting of 5 µl of reverse transcription product, 1 µl of forward primer, 1 µl of downstream primer, 5 µl of reverse transcription product, and 5.5 µl of double distilled water. The primers used were: MRP1 upstream, 5'-GAGGAAACCATATTACAGTGTCGGT-3' and downstream, 5'-AGGGGATCATCGAAGGGTAAAT-3', with a product of 188 bp; MRP2 upstream, 5'-AATAGCACC GACTATCCAGCAT-3' and downstream, 5'-GTGAGAGT GAATTGGGGACCTG-3', with a product of 456 bp; reference of β-actin upstream, 5'-CTTATGGCAGTACACCCCTTTC-3' and downstream, 5'-GTCACCTTACCGTTCCAGT-3', product of 526 bp. The PCR reaction conditions for MRP1 were: 94˚C for 3 min, followed by 33 cycles of 94˚C for 30 sec, 55˚C for 30 sec, 72˚C for 1 min, and 72˚C for 5 min. The PCR reaction conditions for MRP2 were: 95˚C for 2 min, followed by 35 cycles of 95˚C for 30 sec, 56˚C for 30 sec, 72˚C for 1 min, 72˚C for 5 min. The relative expression amount of MRP1 and MRP2 was expressed with the corresponding amplified products and gray level ratio of β-actin.

Analysis of the relative amount of MRP1 and MRP2 protein expression using western blot analysis

The amount of the target β-actin band yielded the relative expression amount of the target protein band. The optical density ratio of the target band/β-actin band yielded the relative expression amount of the target protein band.

Statistical analysis

The results were presented as mean ± SD. The SPSS 16.0 statistical software was applied to compare the expression of MRP2 genes and proteins in all the groups. The ANOVA test was applied to make comparisons between the groups, and the LSD test was applied to make comparisons between any two groups. P<0.05 was considered to indicate statistically significant results.

Results

Expression of mRNA of MRP1 and MRP2

The relative expression amount of mRNA of MRP1 and MRP2 in the IE group was compared to the group of children with epilepsy controlled by anti-epileptic drugs and the group of healthy children without epilepsy. MRP, multidrug-resistant associated protein.
increased compared to the AEDs group and the group comprising healthy children without epilepsy. The difference was statistically significant (P<0.001). However, the relative expression of mRNA of MRP1 and MRP2 in the AEDs group compared to the group of healthy children without epilepsy was not statistically significant (P>0.05) (Table I and Figs. 1 and 2).

Relative expression amount of protein of MRP1 and MRP2. The relative expression of proteins MRP1 and MRP2 in the IE group increased compared to AEDs group and the group of healthy children without epilepsy. The difference was statistically significant (P<0.001). However, a comparison of the relative expression amount of proteins MRP1 and MRP2 in the AEDs group with that in the group of healthy children without epilepsy indicated no statistically significant difference (P>0.05) (Table II and Fig. 3).

Discussion

The mechanism of resistance for intractable epilepsy remains to be elucidated. Findings of recent studies have shown that overexpression of the multidrug transporter may be one of the factors for this resistance (11-13). MRP is an important multidrug transporter that is involved in adjusting the density of antiepileptic drugs within and beyond the cells of epileptic individuals (14,15).

Although significant progress has been revealed in MRP structure and function studies, the majority of these mainly focus on tumors and blood diseases (16,17). Investigations into
the expression of MRP are relatively few and limited to animal epileptic models or in vitro adult brain tissue samples (18-20), which restricts the progress of studies due to difficulty in sampling brain tissues and repeating, as well as small sample size. However, collection from peripheral blood is simple and convenient, and may support dynamic observation. In addition to brain tissues, the expression of MRP also exists in the respiratory tract, digestive tract, urinary tract, and peripheral blood (8,21). Antiepileptic drugs initially enter the bloodstream and then the brain tissues through blood brain barrier. The concentration of drugs with pharmacological effects are similar to the free concentration in blood. Thus, MRP may be induced to overexpress simultaneously inside the blood and brain.

The expression of mRNA of MRP increased significantly in the peripheral blood of recurrent acute leukemic patients and MDR-TB patients (9,22) which indicated that a high expression of MRP in peripheral blood was closely associated with the recurrence of leukemia and multidrug resistance of tuberculosis. In addition, it has been identified that the expression of MDR genes and MRPI mRNA in the peripheral blood of IE adult patients was significantly higher than that of the group controlled by anti-epileptic drugs and the healthy control group, which indicated that MDR1 and MRPI may be associated with the tolerance of epilepsy (23,24). However, the number of studies focusing on MRP expression in peripheral blood of epileptic children, especially IE children, are limited. Thus, in the present study, we concentrated on examining the expression of the MRP gene and protein in peripheral blood mononuclear cells of IE children, to discuss the role of MRP in the pathogenesis of IE children. The result showed that MRPI and MRP2 was expressed in the peripheral blood mononuclear cells of, not only IE children, but also the AEDs children and the healthy children without epilepsy (25,26). Compared to the AEDs and the children without epilepsy, a higher mRNA and protein expression of MRPI and MRP2 for IE children was observed, and the difference was statistically significant. By contrast, no difference was identified between the AEDs group and the healthy control group (27). The results indicate that MRPI and MRP2 was distributed extensively in the peripheral blood of the different groups. Of note, single drug induction did not cause the increase of MRPI and MRP2 in peripheral blood, thus, MRPI and MRP2 may be involved in the resistance process of the IE group. This result was consistent with the findings of Lan et al following an investigation of the MRPI in the peripheral blood of adult IE patients (28).

Repeated abnormal discharges of the neurons in the brain and epileptic seizures are considered the major induction factor of a high MRP expression. For example, studies on rat in the status epilepticus have shown that the expression of MRPI and MRP2 in neurons in the hippocampus and surrounding cortex (29), vascular endothelial cells as well as astrocyte increased significantly. The long-term intervention treatment of certain anti-epileptic drugs, such as oxcarbazepine, may also induce the expression of MRPI in rat (30). However, investigations into tuberous sclerosis identified that the expression of MRPs in certain patients already existed prior to the treatment of anti-epileptic drugs (31).

The results of the present study have shown that the application of anti-epileptic drugs failed to increase MRPI and MRP2 in the peripheral blood of the AEDs. This finding indicates that besides the effects of repeated epileptic seizure and anti-epileptic drugs, elevation of MRP may also be the result of multiple factors and mechanisms, such as the types and acting time of the influential factors, including genetics and immunity. Additionally, the polymorphism and haplotype of MRP genes may affect the reactions of epileptics towards anti-epileptic drugs, thereby resulting in IE. Therefore, the exact mechanism of MRP in IE tolerance requires intensive and extensive investigations.

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