patient with non-paraneoplastic limbic encephalitis as presentation of X-linked lymphoproliferative disease. In that patient, there was no signaling lymphocytic activation molecule-associated protein, which plays a critical role in the regulation of cell signaling in NK cells and T cells. However, autoantibodies were not examined in the patient.

It has become evident that NK cell dysfunction is involved in the pathophysiology of autoimmune diseases, including multiple sclerosis and systemic lupus erythematosus [7]. Several possible mechanisms can be considered [7]. First, defective NK cell responses to viral infections may lead to uncontrolled infections, which cause excessive tissue destruction and thereby subsequent exposure of self antigens. Second, NK cells modulate autoreactive responses of T and B cells directly through the release of cytokines or indirectly through bidirectional interactions with other components of the innate immune system. Experimental studies have shown that NK cell depletion in mouse models enhances the development of autoantibody-secreting B cells [7]. Lastly, NK cells could potentially mediate an autoimmune response by inappropriately killing normal tissues. Thus, it seems possible that impairment of NK cell function could have contributed to the production of GluR autoantibodies in the present patient. The increased proportion of CD 20 lymphocytes might have been due to proliferation of activated B cells, which secrete these autoantibodies.

The present case also showed that hypercytokinemia may play an important role in the pathophysiology of AERRPS. The elevated plasma concentrations of proinflammatory cytokines, such as IL-6 and TNF-α, accompanied by the elevated plasma level of anti-inflammatory IL-10, indicated the existence of inflammation in the blood [8]. In addition, the elevated plasma level of IFN-γ indicated that the causative etiology was most likely viral infection because IFN-γ plays a crucial role in the host defense against viral infections and inhibits viral replication [9]. However, the lack of detection of IFN-γ in the CSF argued against direct invasion of the virus into the CNS. In particular, the present patient had an extremely high CSF concentration of IL-6, without an accompanying anti-inflammatory IL-10 response. The most likely explanation for this finding is that it might have reflected the neuroprotective role of IL-6 against the ischemic and excitotoxic damage induced by repetitive partial seizures [10].

The clinical significance of NMDA-type GluR autoantibodies has not yet been established [11]. Although antibodies against the GluRε2 subunit are positive in some AERRPS patients, these autoantibodies can be identified in a wide variety of disorders, including Rasmussen's encephalitis or progressive epilepsia partialis continua, and autoimmune limbic encephalitis [11]. Notably, the present patient also had IgG antibodies against the Gluζ1 and δ2 subunits. Similarly, however, these autoantibodies appear to have no disease specificity because the former antibodies are identified in patients with paraneoplastic encephalopathies [11], and the latter are detected in those with acute cerebellitis [12] Nevertheless, the demonstration of multiple GluR autoantibodies probably indicated abnormally high degrees of autoimmunity elicited in this patient, which might have been related to the underlying NK cell dysfunction.

In conclusion, the present case suggested that AERRPS is parainfectious immune-mediated encephalitis probably due to the production of excess cytokine and NMDA-type GluR autoantibodies. NK cell dysfunction may be the underlying abnormality in some patients with AERRPS, being responsible for the development of autoimmunity. Therefore, treatment should be targeted towards rapid resolution of hypercytokinemia and prompt clearance or reduction of early generated autoantibodies. Further studies are warranted to confirm our findings, and to reveal the pathogenic significance of the GluR autoantibodies in AERRPS.

Conflict of interest

The authors report no conflict of interest.

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Original article

Open study of pranlukast add-on therapy in intractable partial epilepsy

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Abstract

Innovative treatments of epileptic seizures are needed to improve the outcome of epilepsy. We studied the effect of pranlukast on seizure outcome in patients with intractable partial epilepsy. An open study was conducted to evaluate the clinical efficacy of 24-week pranlukast add-on therapy in 50 patients with intractable partial seizures. Serum concentrations of matrix metalloproteinase (MMP)-9 were determined using Biotrak Activity Assay System. Cytokines in cerebrospinal fluid (CSF) were measured by the Bio-Plex (BioRad) system and soluble TNF receptor1 (sTNFR1) in CSF was measured by the ELISA. Surface markers of lymphocytes in CSF were examined by cell-sorter. Seizure-free rate (SFR) was 13.6%, responder rate (RR) was 47.7%, and aggravation rate (AR) was 18.2% at the 13–24 week period after starting pranlukast. In patients with increased serum MMP-9 before pranlukast therapy (baseline), comparison of paired serum levels showed a significant decrease after pranlukast therapy. Baseline CSF levels of IL-1β and IL-6 were elevated in patients compared with disease controls. Of four patients with paired data, three (including a responder to pranlukast) showed decreased pro-inflammatory cytokines (IL-1β, IL-6, and TNFα), and four showed decreased sTNFR1, after pranlukast treatment, and only a responder had markedly decreased frequency of CD8+ T cells in CSF. Pranlukast reduces seizure frequencies probably by pleiotropic effects including normalization of MMP-9 in sera, reduced leakage of pro-inflammatory cytokines into CNS, and inhibition of extravasation of leucocytes from brain capillaries. Further investigations by double-blind control study and animal models are warranted.

Keywords: Pranlukast; Epilepsy; Matrix metalloproteinase-9; Cytokines

1. Introduction

Epilepsy is a common neurological disease [1], and has variable seizure and cognitive outcomes. Seizure-free outcome was obtained in 47% of patients in response to the first antiepileptic drugs (AEDs), and in 13% in response to the second AEDs, with a total of 64% of patients subsequently achieving freedom from seizures [2]. These data indicate that 36% of patients with intractable seizures are not adequately controlled by conventional AEDs, and that the second or the third AEDs are less likely to be effective in patients whose seizures are not controlled by the first AEDs. Conventional AEDs have similar antiepileptic actions such as inhibition of excitatory ion channels and augmentation of inhibitory ion channels expressed around synapses [3]. Hence, there is a need to develop innovative treatments for epileptic seizures to improve the outcome of

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epilepsy, or even to prevent the onset of epilepsy after the causative brain insults.

The development of epileptic seizures involves epileptogenic and ictogenic processes. After brain insults, several biological changes occur leading to the onset of epilepsy, and these processes are called epileptogenesis. For example, neuronal and interneuronal death, reactive gliosis, reorganization, and aberrant dentate neurogenesis are some reported epileptogenic processes [3]. In human epileptic brain with focal cortical dysplasia (FCD), increased co-assembled expression of N-methyl-D-aspartate type glutamate receptor (NR)2B or NR1 with postsynaptic density protein (PSD)-95 may underlie a cellular epileptogenic mechanism that contributes to in situ increased hyperexcitability, leading to epileptic seizure generation [4]. On the other hand, the biological processes that generate each seizure are called ictogenic processes, and transiently increased glutamate level in synaptic regions has been reported as one of the mechanisms [5]. Drugs targeting epileptogenesis are called anti-epileptogenics, and those targeting ictogenesis include AEDs and anti-convulsants. As far as we know, anti-epileptogenics have not been launched for the treatment of human epilepsy, although clinical trials of topiramate and levetiracetam are ongoing [6]. Epilepsies of various etiologies may be associated with inflammation resulting from increased levels of inflammatory mediators in the brain, and inflammatory mediators can be produced by neurons, glia, and endothelial cells in the blood–brain barrier (BBB) [7]. Inflammation in brain might contribute to the onset and perpetuation of seizures in a variety of epilepsies [7]. New AEDs targeting inflammation are expected to inhibit ictogenesis and epileptogenesis.

Pranlukast (ONO-1078), a cysteinyl leukotriene receptor 1 antagonist (CysLTR1A), has been used for the treatment of bronchial asthma, because pranlukast inhibits smooth muscle contraction and vascular permeability [8]. Studies published in 2009 and 2011 have demonstrated that pranlukast protects endothelial cells from ischemic injury in a leukotriene-independent manner, and this effect results from decreased reactive oxygen species (ROS) levels by ameliorating antioxidant enzyme activity [9,10]. In another recent report, chronic administration of pranlukast, but not acute administration, inhibited pentylenetetrazole (PTZ)-induced convulsions and kindling in rats [11].

In 2004, we encountered a child with intractable partial epilepsy after acute encephalitis. We prescribed pranlukast for the treatment of concurrent bronchial asthma, and his seizures were reduced gradually from 50 times to four times per day. Thereafter, we started an open study of pranlukast add-on therapy in patients with intractable partial epilepsy, with a protocol approved by the ethical committee of our hospital. We evaluated the seizure outcome, and also examined the mechanism of seizure reduction by pranlukast by evaluating matrix metalloproteinase-9 (MMP-9) in serum as well as pro-inflammatory cytokines and T-cell markers in cerebrospinal fluid (CSF).

2. Methods

2.1. Patients and protocol

Sixty-three epileptic patients with intractable partial seizures were enrolled in this study, independent of association of allergic diseases and seizure frequencies. Pranlukast was prescribed from a starting dose of 7 mg/kg/day in children or 225 mg/day in adults, and increased if necessary to a maximum of 10 mg/kg/day in children or 450 mg/day in adults, depending on the seizure frequency. Seizure frequencies were recorded by guardians and patients in seizure diaries. Patients visited our centre in principle every four weeks, the same as before the pranlukast study, and data of seizure frequency for the period between two visits were entered into medical records. Efficacy of pranlukast add-on therapy was evaluated up to 24 weeks of treatment.

Of 63 patients enrolled initially, ten were excluded from analysis because of uncountable seizures (including frequent nocturnal seizures, epilepsia partialis continua, and nonconvulsive status), two were excluded due to the lack of seizure frequency data for the first 12 weeks after study initiation, and one was excluded due to the addition of another AED. Among the 50 patients analysed, prescriptions of AEDs were not changed during the course of the pranlukast study in 32 patients, and AEDs considered to be ineffective before the open study were reduced in nine patients. In the remaining nine patients, doses of AEDs were increased and only three showed decrease in seizure frequency at 12 weeks from the start of the open study. Efficacy of pranlukast was already confirmed before AED doses were increased in two patients, and zonisamide (ZNS) was increased from 100 to 110 mg/day without increase in serum ZNS level in one patient. Montelukast was not prescribed.

2.2. Evaluation of antiepileptic effect of pranlukast

From the data of seizure frequencies recorded for each observation period of four weeks, we calculated the seizure-free rate, 50% seizure reduction rate (responder rate, RR), and aggravation rate (AR). Baseline seizure frequencies were recorded among 12 weeks before the open study. Seizure-free rate (SFR) was defined as the proportion of patients without seizures in the preceding four weeks of each visit. RR was defined as the proportion of patients with 50% reduction of seizures compared to baseline seizure frequencies. AR was defined as the proportion of patients with seizures greater than 1.5 folds compared to baseline seizure frequencies. Long-term antiepileptic effect of pranlukast was evaluated by the

comparison between baseline frequencies and frequencies from 13–24 weeks of the open study.

2.3. Biochemical and immunological markers

Serum concentrations of MMP-9 were determined using MMP-9 Biotrak Activity Assay System (RPN2634; GE Healthcare, Japan) according to the manufacturer's recommendation. The MMP-9 kit measures both the pro- and active-forms of MMP-9. Percentages of CD4+ T cells and CD8+ T cells in CSF were enumerated using a cell-sorter in a commercial laboratory. Immunological markers measured by a BioPlex (BioRad) system were interleukin (IL)-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor (FGF) basic, granulocyte colony-stimulating factor (CSF), granulocyte macrophage CSF, interferon (IFN)-γ, IFN-γ-inducible protein (IP)-10, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor (PDGF)β, RANTES, tumour necrosis factor (TNF)α, and vascular endothelial growth factor (VEGF). Soluble TNF receptor 1 (sTNFR1) was determined by an ELISA kit (Cosmo Bio, BMS03).

2.4. Statistical analyses

Statistical analyses were performed with Mann Whitney test and paired *t* test. Data are shown as mean (95% confidence interval).

3. Results

Clinical efficacy of pranlukast for epileptic seizures was evaluated in 50 patients (male, 32; female, 18) comprising 33 children (younger than 15 years of age) and 17 adolescents or adults (15 years of age or older). The etiology of epilepsy was acute encephalitis in 22, perinatal insults in five, infection in four, FCD in two, others or unknown in 17 patients. Infectious etiology was defined as onset of epilepsy shortly after a mild infection without involvement of the central nervous system (CNS). Mean (95% confidence interval) onset age was 7.4 (5.1–9.7) years, mean age at the start of pranlukast was 13.2 (10.1–16.2) years, and mean interval from onset of epilepsy to start of pranlukast was 5.8 (4.2–7.4) years. Mean seizure frequency among 12 weeks before adding pranlukast was 3.9 (2.3–5.5) seizures/day. Mean number of AEDs at the start of pranlukast was 2.1 (1.9–2.4): valproate (VPA) was prescribed in 16 patients, carbamazepine (CBZ) in 15, ZNS in 12, phenytoin (PHT) in 23, and phenobarbital (PB) in 17. Mean dose of pranlukast at 12 weeks was 178.3 mg (137.4–219.1) in children, and 352.3 mg (299.3–405.4) in adults.

The number of patients evaluated for each observational period differed due to various factors such as uncontrolled visiting intervals and cessation of pranlukast (Fig. 1). During the 24-week treatment, pranlukast was discontinued because of lack of efficacy in three patients (6%) and general fatigue in one (2%). The mean dose of pranlukast was 199.0 mg/day at four weeks, 223.3 mg/day at eight weeks, 238.8 mg/day at 12 weeks, 243.5 mg/day at 16 weeks, 253.9 mg/day at 20 weeks, 254.0 mg/day at 24 weeks, and 258.2 mg/day at 24 weeks. The dose of pranlukast was increased in 14 of 50 patients during the second period (5–8 weeks), in seven during the third period (9–12 weeks), in five patients during the fourth period (13–16 weeks), and in nine patients after 17 weeks.

The SFR was 23.9% at the 9–12 week period, and was 13.6% at the long-term observation period (13–24 week). At the 9–12 week period, seven patients aged below 15 years and four patients aged 15 years or above (total 11, 23.9%) were seizure-free. Subsequently, six of the seven seizure-free children (85.7%) remained seizure-free at the final observation of 24 weeks. On the other hand, only one of four (25.0%) seizure-free older patients remained seizure-free at the final observation. After 13 weeks, two novel patients of childhood became seizure-free in response to increased dose of pranlukast. In 32 patients without change of prescriptions of AEDs among the first 12 weeks of the open study, the SFR was 27.6% at the 9–12 week period.

The RR was 47.7% at the long-term observation period (13–24 week). At the 9–12 week period, 12 patients aged below 15 years and 8 patients aged 15 years or above (total 20 of 46 patients) were responders. Subsequently, 10 of 12 (83.3%) responding younger patients remained responders at the final observation of this study. On the other hand, four of eight (50.0%) responding older patients remained responders at the final observation. After 13 weeks, seven novel patients of childhood and three novel patients aged 15 or above became responders and remained so until 24 weeks. In 32 patients without change of prescriptions of AEDs among the first 12 weeks of the open study, the RR was 55.2% at the 9–12 week period.

The AR was 21.7% at the 9–12 week period, and was 18.2% at the long-term observation period (13–24 week). At the 9–12 week period, seven patients aged below 15 years and three patients aged 15 years or above (total ten of 46 patients) were aggravated. Subsequently, two of seven (28.6%) aggravated younger patients remained aggravated at the final observation, one became seizure-free in response to increased dose of pranlukast, and two became responders. On the other hand, one of three (33.3%) aggravated older patients remained aggravated at the final observation, and two resumed the seizure frequencies before the pranlukast study. After 13 weeks, three novel adult patients became aggravated and remained so until 24 weeks. In 32 patients without change of prescriptions of AEDs among the first

12 weeks of the open study, the AR was 20.7% at the 9–12 week period.

The relationship between efficacy of pranlukast and etiology of epilepsy was evaluated using data of the 9–12 week period, because doses of pranlukast were increased in many patients from five to eight weeks after starting this study. Patients who developed epilepsy after acute encephalitis had lower SFR but almost the same RR compared to those with epilepsy after perinatal insults. In patients with post-acute encephalitis epilepsy, the mean seizure reduction rate at the 9–12 week period compared to before study was not different between patients younger than 15 years and those 15 years or older (data not shown, $p = 0.79$). The relationship between efficacy of pranlukast and age of epilepsy onset was evaluated using data of the 9–12 week period, and showed no definitive findings. The relationship between efficacy of pranlukast and age at pranlukast therapy was evaluated using data of the 9–12 week period. In patients aged below 15 years, SFR was higher when pranlukast was started at a younger age. The relationship between efficacy of pranlukast and interval between epilepsy onset and pranlukast treatment was evaluated using data of the 9–12 week period. Both SFR and RR were the highest and AR was the lowest when the interval was the shortest (0–1.4 years).

Mean serum MMP-9 level was 81.13 (45.96–116.30) ($n = 21$) ng/ml before pranlukast treatment and 56.90 (39.95–73.85) ($n = 24$) ng/ml after pranlukast treatment in the patient subjects, and was 49.61 (43.56–55.65) ($n = 30$) ng/ml in healthy controls (Fig. 2A). A significant difference was detected only between patients before pranlukast treatment and healthy controls ($p = 0.0298$). Statistical comparison of paired samples from the same patients collected before and after pranlukast treatment ($n = 19$) showed significant difference ($p = 0.0156$) (Fig. 2B). When the same comparison was conducted on patients with increased MMP-9 levels (higher than mean levels of healthy controls) before pranlukast treatment ($n = 15$), a significant difference was detected ($p = 0.0133$) (Fig. 2C). Rates of seizure frequency between 13–24 week period and baseline (13–24 week period/baseline) tended to be lower in patients with higher rates of MMP-9 reduction ({MMP-9 before – MMP-9 after}/MMP-9 before) (Fig. 2D). After pranlukast treatment, mean serum MMP-9 level was 38.74 (17.53–59.95) ($n = 8$) ng/ml in patients with seizure frequency below weekly levels, and 87.84 (2.44–173.20) ($n = 5$) ng/ml in patients with daily seizures (Fig. 2E) ($p = 0.0295$).

Mean cell count in CSF was 1.647 (1.013–2.282)/mm^3 ($n = 19$) before and 1.310 (0.4708–2.149)/mm^3 ($n = 8$) after pranlukast treatment in patient subjects, and was 1.234 (0.8492–1.620)/mm^3 ($n = 87$) in disease controls. A significant difference was detected only between patients before pranlukast treatment and disease controls ($p = 0.0121$). Mean protein level in CSF was 27.42 (21.16–33.68) mg/dl ($n = 19$) before and 22.68 (11.89–33.86) mg/dl ($n = 8$) after pranlukast treatment in patient subjects, and was 20.35 (19.04–21.66) mg/dl ($n = 84$) in disease controls. No significant difference was observed among three groups.

Mean frequency of CD4+ T cells in CSF was 50.61 (44.74–56.47)% ($n = 21$) before and 58.65 (52.38–64.92)% ($n = 24$) after pranlukast treatment in patient subjects, and was 45.68 (41.59–49.71)% ($n = 30$) in disease controls (Fig. 3A). A significant difference was detected only between subjects after pranlukast treatment and disease controls ($p = 0.0084$). Statistical comparison of paired samples collected before and after pranlukast treatment showed no significant difference (Fig. 3B). Mean frequency of CD8+ T cells in CSF was 23.29 (24.97–39.62)% ($n = 16$) before and 30.74 (25.79–35.68)% ($n = 8$) after pranlukast treatment in patient subjects, and was 27.60 (25.30–29.90)% ($n = 45$) in disease controls (Fig. 3C). No significant difference was observed among three groups. Statistical comparison of paired samples collected before and after pranlukast treatment showed no significant difference, but marked decrease in frequency of CD8+ T cells in CSF was observed only in a responder to pranlukast (Fig. 3D).

Mean level of IL-1β in CSF was 0.59 (0.31–0.86) ($n = 17$) pg/ml before and 0.52 (0.12–0.91) ($n = 6$) pg/ml after pranlukast treatment in patient subjects, and was 0.12 (0.07–0.16) ($n = 39$) pg/ml in disease controls (Fig. 4A). A significant difference was detected between patient subjects before pranlukast treatment and disease controls ($p = 0.0007$) and between patient subjects after pranlukast treatment and disease controls ($p = 0.0116$). Comparing paired samples collected before and after pranlukast, three of four paired data including one

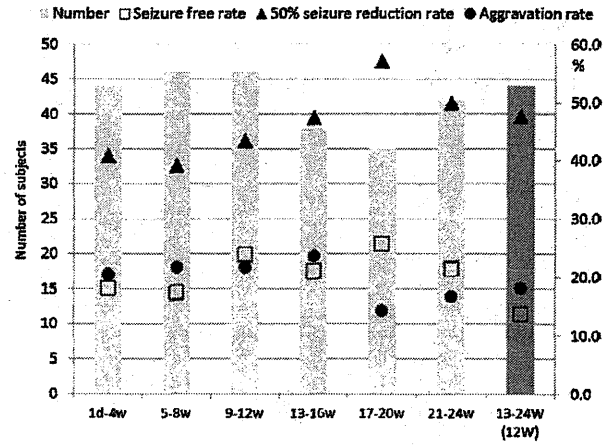

Fig. 1. Efficacy of pranlukast in controlling epileptic seizures. Column shows the number of patients whose seizure frequencies were evaluated in each period. Blank square denotes seizure-free rate (%). Black triangle denotes 50% seizure reduction rate (responder rate) (%). Black circle denotes aggravation rate (%).

responder showed decreased IL-1β levels after pranlukast treatment (Fig. 4B).

Mean level of IL-6 in CSF was 5.83 (1.25–7.41) ($n = 17$) pg/ml before and 4.25 (2.52–5.98) ($n = 6$) pg/ml after pranlukast treatment in patient subjects, and was 3.29 (2.73–3.85) ($n = 39$) pg/ml in disease controls (Fig. 4C). A significant difference was found between patient subjects before pranlukast and disease controls ($p = 0.0009$). Comparing paired samples collected before and after pranlukast, three of four paired data including one responder showed decreased IL-6 levels after treatment (Fig. 4D).

Mean level of TNF-α in CSF was 45.10 (27.15–63.04) ($n = 17$) pg/ml before and 22.14 (0.33–43.95) ($n = 6$) pg/ml after pranlukast treatment in patient subjects, and was 27.19 (22.39–31.98) ($n = 39$) pg/ml in disease controls (Fig. 4E). Comparing paired samples before and after pranlukast, three of four paired data including one responder showed decreased TNF-α levels after pranlukast treatment (Fig. 4F).

Mean level of sTNFR1 in CSF was 0.51 (0.40–0.62) ($n = 14$) ng/ml before and 0.49 (0.32–0.67) ($n = 6$) pg/ml after pranlukast treatment in patient subjects, and was 0.58 (0.50–0.66) ($n = 37$) ng/ml in disease controls, with no significant differences (Fig. 4G). Comparing paired samples before and after pranlukast, all paired data ($n = 4$) including one responder showed decreased sTNFR1 levels after pranlukast treatment (Fig. 4H).

Mean level of IL-8 in CSF was 41.15 (26.91–55.39) ($n = 17$) pg/ml before and 42.28 (22.19–62.38) ($n = 6$) pg/ml after pranlukast treatment in patient subjects, and was 35.62 (28.45–42.79) ($n = 39$) pg/ml in disease controls, with no significant differences (Fig. 4I). Comparing paired samples before and after pranlukast, three of four paired data including one responder showed decreased IL-8 levels after pranlukast treatment (Fig. 4J).

The CSF levels of other cytokines tested were not different among patient subjects before and after pranlukast treatment and disease controls.

4. Discussion

In our open study of pranlukast in 50 patients with intractable partial seizures, pranlukast was discontinued only in four patients (8%) up to 24 weeks of treatment, due to lack of efficacy (6%) or adverse effect (2%). SFR was 23.9% at the 9–12 week period, and was 13.6% at the long-term observation period (13–24 week). RR was 43.5% at the 9–12 week period, and was 47.7% at the long-term observation period (13–24 week). AR was 21.7% at the 9–12 week period, and was 18.2% at

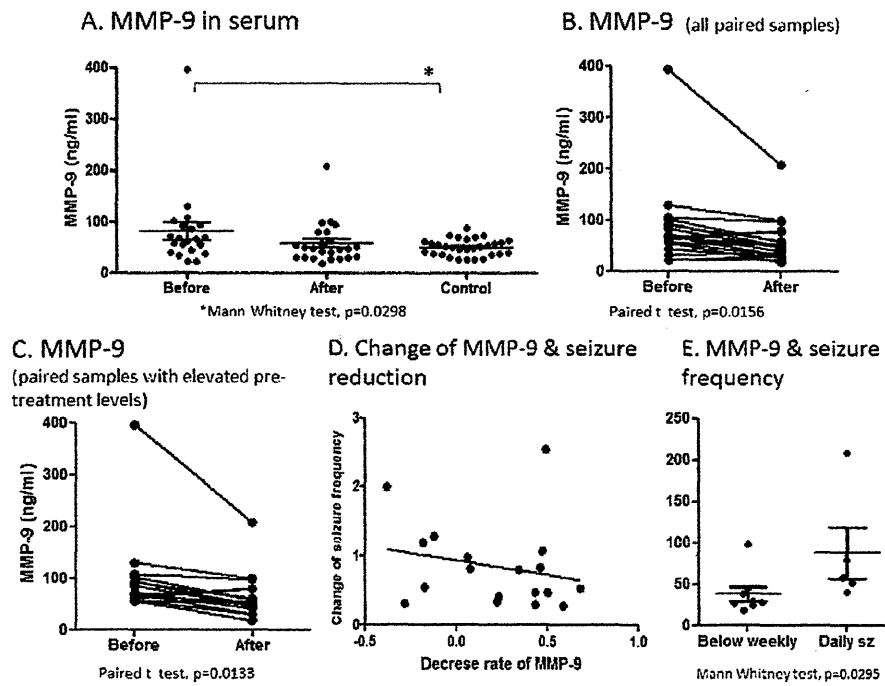

Fig. 2. Serum MMP-9 levels before and after pranlukast treatment: compared with healthy controls (A), comparisons of all paired samples (B), and comparison of paired sera from patients with pre-pranlukast treatment MMP-9 levels higher than the mean level of healthy controls (C). Comparison between rates of seizure frequency between 13–24 week period and baseline (seizure frequency at 13–24 week period/seizure frequency at baseline) and rates of MMP-9 level reduction ({MMP-9 levels before – MMP-9 levels after}/MMP-9 levels before treatment of pranlukast) (D). Comparison of MMP-9 levels between patients with seizures below weekly levels and patients with daily seizures after treatment of pranlukast (E). Horizontal bars show mean and standard error.

the long-term observation period (13–24 week). In a post-marketing survey of lamotrigine (LTG) in 35 paediatric patients with intractable partial seizures at our centre, SFR was 5.7%, RR was 42.9%, AR was 8.6%, and discontinuation rate was 22.9% [12]. Seizure frequencies of patients in the study of LTG were almost same as those in this open study of pranlukast. These data may suggest that pranlukast is as efficacious as LTG for the antiepileptic effect, and that SFR and discontinuation rate of pranlukast surpass those of LTG in patients with intractable partial seizures. In patients with intractable partial epilepsy, RR of topiramate was reported as 39%, RR of oxcarabamazepine 41%, RR of levetiracetam 44.6%, RR of gabapentin 21.2% [13–16]. These data also suggest the efficacy of pranlukast.

The SFR of pranlukast was 13.6% at the long-term observation period in the present study, and the SFRs of conventional AEDs for intractable epilepsy have been reported to be less than 15% [17,18]. However, SFR of pranlukast was higher when the drug was started in patients at younger ages and earlier from onset of epilepsy. These findings suggest that pranlukast may contribute to the inhibition of epileptic mechanisms, which are dominant in young patients shortly after epilepsy onset. Mean RR of pranlukast was 47.7% at the long-term observation period, and is comparable to those of conventional AED which were reported to range from 10% to 40% (minus placebo) [19]. The efficacy of pranlukast was maintained from 12 to 24 weeks in many patients with respect to SFR and RR. Furthermore, only six of 20 responders at the 9–12 week period (30%) became non-responders at the final observation of this study. In comparison, 58% of responders in the TPM trial became non-responders after 30 months of therapy [20]. Tolerance (loss of effect) to benzodiazepines was observed in 27–48% of patients because of down-regulation of target sensitivity or functional uncoupling of target (GABAaR) [21]. Tolerance to pranlukast seems to be less frequent than to benzodiazepines. These data suggest that pranlukast has advantages over conventional AEDs with respect to seizure control and tolerance in patients with intractable partial seizures.

In our study, adverse effect related to pranlukast was observed in only one patient (2%), who discontinued the drug because of general fatigue. No adverse CNS effects (such as somnolence and vertigo) commonly seen with conventional AEDs were found during pranlukast treatment. For conventional AEDs acting on CNS, the discontinuation rates due to adverse reactions range from 10% to 30% [17]. The reported adverse effect rate of pranlukast is 2.9% in patients with allergic diseases (http://www.info.pmda.go.jp/go/pack/4490017R1033_1_07/). These data suggest that pranlukast plays an important role in seizure reduction besides direct action on CNS, and is a safer drug for treating patients with intractable partial seizures.

Our study of serum MMP-9 showed a significant difference before and after pranlukast administration, especially among patients with increased pre-treatment MMP-9 levels (Fig. 2B and C). In patients with larger decrease of MMP-9 after treatment by pranlukast,

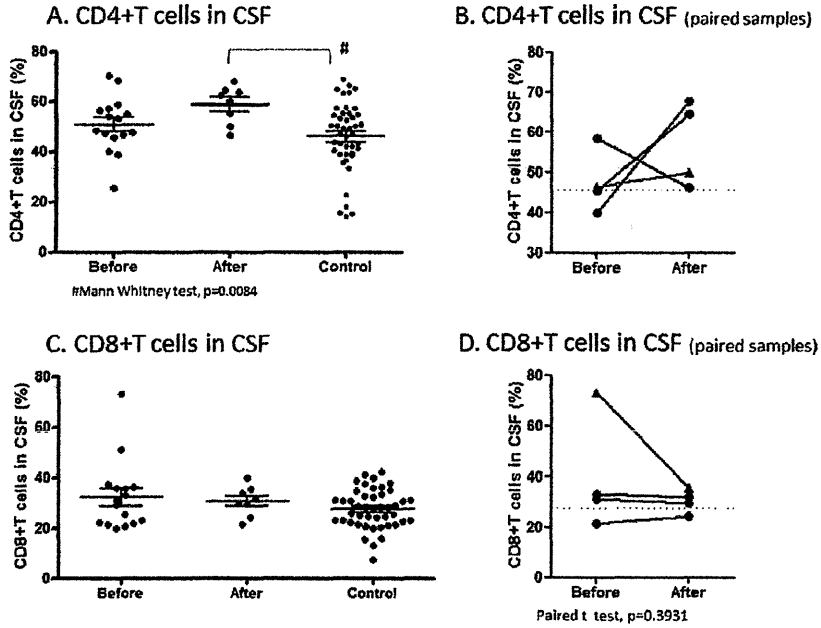

Fig. 3. Frequencies of CD4+ T cells (A) and CD8+ cells (C) in CSF samples of epileptic patients collected before and after pranlukast treatment compared with control patients without inflammatory disease (disease controls), and comparisons of frequencies of CD4+ T cells (B) and CD8+ cells (D) in paired samples collected from the same patients before and after pranlukast treatment. In B and D, triangle denotes responder to pranlukast, in whom seizure frequencies were reduced by more than 50% compared to before open study, and circle denotes non-responder.

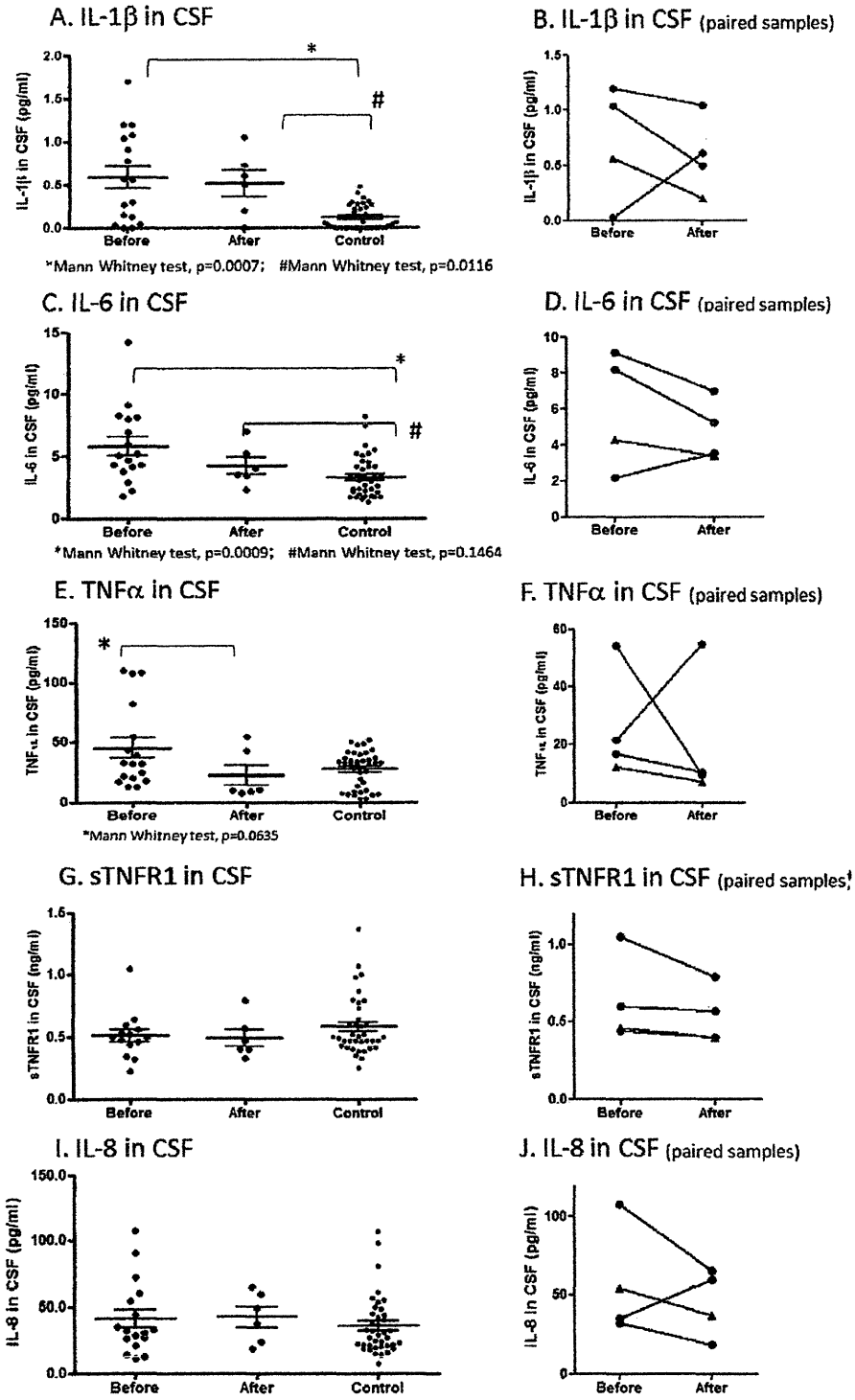

Fig. 4. Cerebrospinal fluid (CSF) cytokine levels in epileptic patients before and after pranlukast treatment and in control patients without inflammatory disease (disease controls): IL-1β (A), IL-6 (C), TNFα (E), sTNFR1 (G), IL-8 (I). Comparison of paired samples collected from the same patients before and after pranlukast treatment: IL-1β (B), IL-6 (D), TNFα (F), sTNFR1 (H), IL-8 (J). In data of paired samples, triangle denotes responder to pranlukast whose seizure frequencies were reduced by more than 50% compared to before pranlukast treatment, and circle denotes non-responder.

seizure frequencies tend to be decreased at greater extent, and levels of MMP-9 were higher in patients with frequent seizures. Cysteinyl leukotriene enhance TNF-α-induced MMP-9 production by human monocytes/macrophages, and pranlukast completely inhibits the enhancement of TNF-α-induced MMP-9 production

by leukotriene (LT) C4 and LTD4 [22]. Furthermore, pranlukast inhibits VEGF production in human monocytes/macrophages, and prevents vascular hyperpermeability [23]. These findings suggest that pranlukast may normalize serum MMP-9 levels and protect BBB function in epileptic patients. Forty-four percentage of patients in this open study had a history of acute encephalitis, and BBB dysfunction in the acute stage of encephalitis is reported to continue until the recovery stage [24].The high proportion of post-encephalitis epileptic patients in this study may account for the better seizure outcome of pranlukast treatment, presumably through normalizing MMP-9 level and BBB function. Normalization of MMP-9 may reduce the leakage of pro-inflammatory cytokines through the BBB.

Pranlukast is known to attenuate hydrogen peroxide–induced necrosis in endothelial cells by inhibiting oxygen reactive species–mediated collapse of mitochondrial membrane potential [9,10], and montelukast (another CysLTR1A) is also known to attenuate chronic brain injury after focal cerebral ischaemia in mice and rats [25]. These experimental data suggest that CysLTR1As have endothelial cell protective effects, and may reduce the leakage of pro-inflammatory cytokines through BBB. Patients of this open study had higher CSF levels of IL-1β and IL-6 before pranlukast treatment compared to disease controls, and comparison of paired samples showed that three (including one responder to pranlukast) of four patients had decreased pro-inflammatory cytokines (IL-1β, IL-6, and TNFα) after pranlukast treatment (Fig. 4). Interleukin-1β activates the N-methyl D-aspartate receptor (NR)2A/NR2B subunits, thereby contributing to glutamic acid-induced neurodegeneration [26]. Interleukin-1β is also known to inhibit glutamic acid uptake by glia and enhance glutamic acid release from glia mediated by TNFα production, leading to elevated glutamic acid concentration in the synaptic gaps and ultimately to neuronal excitation [26]. These findings suggest that IL-1β may contribute to neuronal excitation in epilepsy. High concentrations of TNFα have been shown to increase excitotoxic death of neurons by increasing synaptic AMPA receptors and decreasing GABA receptors [27], and cause spasms in TNFα transgenic mice [28]. Based on these findings, it is possible that TNFα gradually increases neuronal excitability and contributes to epileptogenesis.

CysLTR1As are known to inhibit capillary permeability, and pranlukast only inhibits extravasation of white blood cells from brain capillary [29]. In this open study, cell counts in CSF of epileptic patients before pranlukast treatment were higher than controls, but were not different after treatment. Furthermore, marked decrease in frequency of CD8+ T cells in CSF was observed only in one responder to pranlukast (Fig. 3D). These data suggest that pranlukast inhibits extravasation of white blood cells including CD8+ T cells into the CNS. In a model of pilocarpine-induced status epilepticus, perforin produced by cytotoxic lymphocytes (CD8+ T cells) are key molecular players involved in the axis between peripheral intravascular inflammation and seizures [30]. Therefore, reduction of CD8+ T cells in CNS by pranlukast may impact the seizure frequency.

Pranlukast seems to have pleiotropic effects on epileptic seizures besides direct actions on CNS, including normalization of MMP-9, protection of endothelial cells by inhibiting oxygen reactive species resulting in reduced leakage of pro-inflammatory cytokines into CNS, and inhibition of extravasation of white blood cells from brain capillary. These pleiotropic effects may reduce epileptic seizures with little adverse effects on CNS. Although pranlukast cannot pass through BBB under ordinary conditions, it may pass through damaged BBB in patients after acute encephalitis. If pranlukast reaches CNS in patients with BBB dysfunction, reduction of oxygen reactive species by pranlukast would suppress epileptogenesis and ictogenesis. In an animal study published in 2011, chronic administration of pranlukast but not acute administration of pranlukast inhibited PTZ-induced convulsions and kindling in rats [11]. Furthermore, acute administration of montelukast attenuated the development of seizures in animal models [31].

Our observational study could only show associations of biochemical values vs seizure outcome in intractable epilepsy, but could not show definitive causality in seizure reduction after pranlukast treatment. Further studies are required to examine the antiepileptic and anti-epileptogenic effects of pranlukast through double-blind controlled clinical trials with more strict protocols and animal models.

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.

Disclosure of conflicts of interest

All authors have no conflict of interest to disclose.

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Original article

Immunomodulatory therapy versus surgery for Rasmussen syndrome in early childhood

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Abstract

We examined seizure, cognitive, and motor outcomes in patients with Rasmussen syndrome or Rasmussen encephalitis (RS), after recent initiation of immunomodulatory therapies. Among 53 patients with a diagnosis of RS referred from all over Japan, 49 patients (male 22, female 27) with symptoms and findings characteristic of RS were evaluated. Regular intravenous immunoglobulin (IVIg) therapy was administered at a dose of 100 mg/kg/day, etc. Regular steroid pulse therapy was conducted with methylprednisolone at a dose of 30 mg/kg/day (children) or 1000 mg/day (adults) for 3 days. Tacrolimus was given at an initial dose of 0.1 mg/kg/day (children). Mean onset age was 8.7 ± 10.5 years. Seizure-free rate was 71% after treatment by functional hemispherectomy (FH), and response rate for seizures was 81% by regular steroid pulse therapy, 42% by tacrolimus therapy, and 23% by regular IVIg therapy. Rate of patients with IQ higher than 80 (R80) was 50% by regular steroid pulse therapy, 43% by regular IVIg therapy, 29% by tacrolimus therapy, and 0% by FH. R80 after regular steroid pulse therapy was 100% in patients without MRI lesions, and 37% in those with advanced MRI lesions. Improvement of motor function (paresis) was observed only by immunomodulatory therapy. Motor function was aggravated in 100% of patients treated by FH, 62% by regular IVIg, and 10% by regular steroid pulse therapy. We suggest a new treatment strategy for RS using early immunomodulatory therapy: initiation of regular steroid pulse therapy after early diagnosis indicated by biomarkers, then switching to tacrolimus therapy after several months.

Keywords: Rasmussen syndrome; Steroid-pulse therapy; IVIG therapy; Tacrolimus; Functional hemispherectomy; Seizure outcome; Cognitive outcome; Motor outcome

1. Introduction

Rasmussen syndrome or Rasmussen encephalitis (RS) is a slowly progressive, autoimmune chronic inflammatory disease of the central nervous systems [1–3]. Preceding infection occurring around two weeks before onset is observed in 38% of patients [3]. Histological examination usually shows inflammatory lesions with T cell infiltration. Cytotoxic T cells (CTLs) contribute to the immunopathology of RS [4]. The IFNγ, IL-12, and granzyme B levels in CSF are elevated suggesting immunological involvement, especially in the early stage of the disease [5].

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In RS, the initial symptom is usually intractable partial seizures, and epilepsia partialis continua (EPC) develops in 58.8% of the patients. Soon after the onset of RS, treatment with antiepileptic drugs (AEDs) is usually initiated because partial seizures are predominant symptoms. In a few years after onset, unihemispheric cortical dysfunctions (such as hemiplegia and cognitive deficit) become apparent [6,7]. RS is suspected when unilateral cortical deficit, unihemispheric EEG slowing, and unihemispheric cortical atrophy on MRI appear evolutionally. Before the availability of immunopathology, functional hemispherectomy (hemispherotomy) (FH) was the only treatment to achieve complete control of epileptic seizures. Therefore, in patients with involvement of the non-dominant hemisphere, FH is considered after the appearance of motor deficits. On the other hand, in those with disease involving the dominant hemisphere, immunomodulatory therapies using corticosteroids, intravenous immunoglobulin (IVIg), plasma pheresis (PEX) or immunoabsorption, and tacrolimus have been tried [2].

In considering treatment strategies for RS, comprehensive consideration of seizure outcome, neurological outcome, cognitive outcome, and motor outcome is necessary. In making a decision to undergo FH, the parents of patients with RS desire to achieve complete control of seizures and normal cognitive development at the sacrifice of hemiplegia.

With recent developments of many kinds of immunomodulatory therapies, we compared the treatment results of Japanese RS patients treated by surgery and/or immunomodulatory therapies, by evaluating their seizure, cognitive, and motor outcomes.

2. Methods

2.1. Patients

We identified 53 patients with a diagnosis of RS referred to the National Epilepsy Center from all over Japan between 1991 and 2010, and reviewed them basically according to the European diagnostic criteria for RS (Fig. 1) [2]. Of 53 patients, three patients who had no frequent partial seizures, and eight patients who had no unihemispheric cortical dysfunction were initially excluded from a diagnosis of RS. From the eight patients without unihemispheric cortical dysfunction, seven patients were subsequently diagnosed as having RS based on characteristic histology, elevated granzyme B in CSF, or high intensity lesion on MRI characteristic of RS [2,8]. RS was staged into three MRI categories: no lesion, high intensity lesion, and advanced MRI lesion.

2.2. Evaluation

Seizure outcome was classified according to the change in seizure frequency before and after treatments

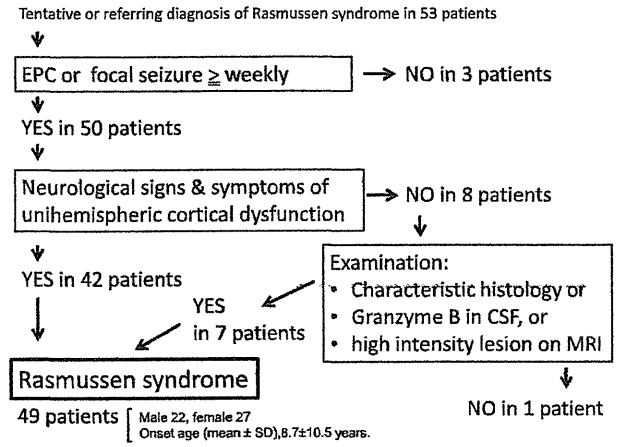

Fig. 1. Patient disposition. EPC, epilepsia partialis continua.

into seizure-free (free), >50% seizure reduction (responder) (decreased), between <50% reduction and <50% increase (stable), >50% seizure increase (aggravated). In patients with EPC and solitary partial seizures, change in frequency of solitary partial seizures was evaluated. Cognitive outcome was measured by intelligence quotient (IQ) or developmental quotient (DQ). IQ was measured by Tanaka–Binet, WISCIII, and WAISIII, dependent on the age at examination. DQ was measured by MCC-baby test, KIDS-test, and other scales. We used full scale IQ (FSIQ) measured by WISC or WAIS for evaluation. Cognitive outcome was classified into FSIQ/DQ increase >10 (improved), between <10 increase and <10 decrease (stable), FSIQ/DQ decrease >10 (aggravated), and uncertain (uncertain). Rate of FSIQ/DQ preservation was calculated as number of patients (improved + stable)/number of patients (improved + stable + decreased). Motor outcome was classified into improved, stable and aggravated.

2.3. IVIg therapy

The protocol for regular IVIg therapy was a dose of either 100 mg/kg/day for several days, 400 mg/kg/day for several days, or 1 g/kg for one day, at an interval of once a month for several months to several years depending on response.

2.4. Steroid pulse therapy

The protocol for regular steroid pulse therapy with methylprednisolone was doses ranging from 30 mg/kg/day (for children) to 1000 mg/day (for adults) for 3 days, at an interval of once in a month for several months to several years depending on response. Only patients who had received more than 3 cycles were evaluated.

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2.5. Tacrolimus therapy

The usual protocol for tacrolimus therapy was a starting dose of 0.1 mg/kg/day (for children) or 3 mg/day (for adults) with dose escalation after 2 months, depending on blood levels of tacrolimus. Only patients who had received treatment for more than 6 months were evaluated.

2.6. Statistical analyses

Non-parametric Mann–Whitney *U*-test was used to compare the quantitative variables between two groups. Chi-square test for trend was used to compare the seizure outcome. A p value <0.05 was considered as indicating a significant difference.

3. Results

3.1. Patients background

Mean onset age of epilepsy in 49 patients (male 22, female 27) was 8.7 ± 10.5 years. Twelve patients had preceding infection, seven had preceding vaccination, and four had preceding head trauma before onset of epilepsy. Dominant hemispheres were involved in 24 patients, and non-dominant in 25 patients.

Regular IVIg therapy was evaluated in 13 patients (dominant side, 7; non-dominant side, 6) (Table 1). Mean onset age was 13.6 ± 16.3 years, and mean lag period from onset to IVIg therapy was 4.0 ± 5.7 years. Regular steroid pulse therapy was evaluated in 21 patients (dominant side, 12; non-dominant side, 9). Mean onset age was 8.2 ± 11.7 years, and mean lag period from onset to steroid pulse therapy was 5.7 ± 6.2 years. Tacrolimus therapy was evaluated in 12 patients (dominant side, 9; non-dominant side, 3). Mean onset age was 8.8 ± 10.4 years, and mean lag period from onset to tacrolimus therapy was 6.4 ± 7.2 years.

Of 49 patients, 30 patients had received at least one kind of immunotherapy during the course of treatment. In these patients, cognitive outcome (the last IQ) was not related to onset age, treatment lag period, or disease duration.

Selection of treatments was determined by the attending doctors (Table 1). Among 24 patients with dominant hemisphere involvement, nine received regular pulse therapy, seven had regular IVIg therapy, and three underwent surgery as the initial therapy, in addition to AED therapies. In 25 patients with non-dominant hemisphere involvement, six had regular pulse therapy, five received regular IVIg therapy, and nine underwent surgery as the initial therapy, in addition to AED therapies. A total of 12 patients were treated with tacrolimus, 11 of whom received tacrolimus as a replacement of regular IVIg or pulse therapy.

3.2. Seizure outcome

Seizure-free rate (SFR) was 71% in patients who underwent FH of the non-dominant hemisphere, 20% surgical resection in the non-dominant hemisphere, and 0% surgical resection in the dominant hemisphere

Table 1
Treatment flow.

Involved hemisphere	1st Treatment	2nd Treatment	3rd Treatment	Number of patients
Dominant: 24 patients	Regular pulse therapy	Regular pulse		3
		Tacrolimus		5
		Surgery	Regular pulse-tacrolimus-regular pulse	1
	Regular IVIg therapy	Regular IVIg		3
		Regular pulse		1
		Tacrolimus		1
		Tacrolimus	Regular pulse	1
		Surgery		1
	Tacrolimus therapy	Regular pulse		1
	Surgery			3
	AEDs only			3
	Others			1
Nondominant: 25 patients	Regular pulse therapy	Regular pulse		3
		Tacrolimus		2
		Regular IVIg		1
	Regular IVIg therapy	Regular IVIg		1
		Regular pulse	Surgery	1
		Tacrolimus	Surgery	1
		Surgery		2
	Surgery	Surgery		7
		Regular pulse		2
	AEDs only			3
	Others			2

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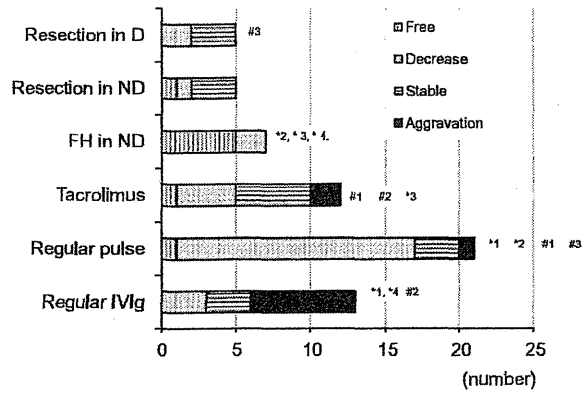

Fig. 2. Seizure outcome after surgery or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of seizure outcome. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. Chi-square test for trend detected significant differences in seizure outcome between two groups marked by (*) (*1, $p = 0.0003$; *2, $p = 0.0023$; *3, $p = 0.0033$; *4, $p = 0.0021$), and non-significant differences between two groups marked by (#) (#1, $p = 0.3080$; #2, $p = 0.2036$; #3, $p = 0.1646$).

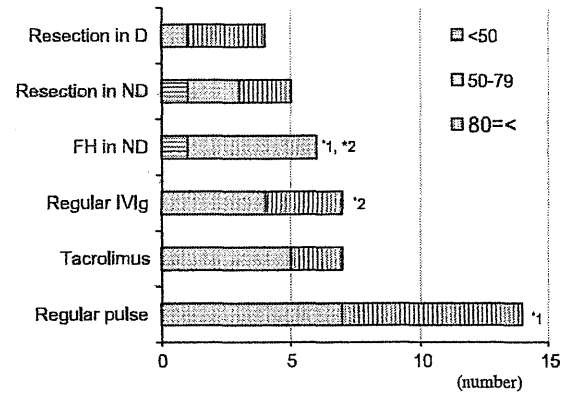

Fig. 3. The last FSIQ/DQ after surgeries or immunomodulatory therapies. Horizontal axis shows the number of patients with each category of FSIQ/DQ. Resection in D, surgical resection in dominant hemisphere; ND, non-dominant hemisphere; FH, functional hemispherectomy; IVIg, intravenous immunoglobulin. Chi-square test for trend detected significant differences in seizure outcome between two groups marked by (*) (*1, $p = 0.0141$; *2, $p = 0.0447$).

(Fig. 2) (Table 2). In two of seven patients treated by FH, seizures relapsed at three and six years after FH. SFR was 8% by tacrolimus therapy, 5% by regular pulse therapy, and 0% by regular IVIg therapy. Greater than 50% reduction rate (response rate, RR) was 81% by regular pulse therapy, 42% by tacrolimus therapy, and 23% by regular IVIg therapy. FH of the non-dominant hemisphere had better seizure outcome compared with regular pulse therapy ($p = 0.0023$), tacrolimus therapy ($p = 0.0033$) and regular IVIg ($p = 0.0021$). Seizure outcome by regular pulse therapy was better than by regular IVIg ($p = 0.0003$), but was not different from tacrolimus therapy or resection in dominant hemisphere.

3.3. Cognitive outcome

We compared the changes in FSIQ/DQ before and after various treatment modalities. Preservation of cognitive function was defined as "improved" and "stable" changes in FSIQ/DQ. Preservation rate of FSIQ/DQ was 76% by regular pulse therapy, 75% by tacrolimus therapy, 60% by surgical resection in non-dominant hemisphere, 60% by surgical resection in dominant hemisphere, 57% by FH of non-dominant hemisphere, and 45% by regular IVIg therapy (data not shown). The changes in FSIQ/DQ before and after treatment were not significantly different among the treatment modalities.

Next, cognitive outcome among the various treatment modalities was compared by the last FSIQ/DQ (Fig. 3). The proportion of patients with FSIQ/DQ higher than 80 after therapy (R80) was 75% by surgical resection in dominant hemisphere, 50% by regular pulse therapy, 43% by regular IVIg therapy, 40% by surgical resection in non-dominant hemisphere, 29% by tacrolimus therapy, and 0% by FH of non-dominant hemisphere. Regular pulse therapy had significantly better FSIQ/DQ than FH of non-dominant hemisphere. Regular IVIg also had significantly better FSIQ/DQ than FH of non-dominant hemisphere.

Table 2
Summary of outcome in Rasmussen syndrome.

		Epileptic surgery			Regular IVIg	Regular pulse	Tacrolimus
		FH in ND	Res in ND	Res in D			
Number		7	5	5	13	21	12
Seizure outcome	SFR (%)	71	20	0	0	5	8
	RR (%)	100	40	40	23	81	42
Cognitive outcome	PR (%)	57	60	60	45	76	75
	R80 (%)	0	40	75	43	50	29
Motor outcome	AR (%)	100	0	20	62	10	0
Discontinuation (%)					100	62	17

FH, functional hemispherectomy; ND, non-dominant hemisphere; Res, resection surgery; D, dominant hemisphere; IVIg, intravenous immunoglobulin; SFR, seizure free rate; RR, response rate; PR, FSIQ/DQ preservation rate; R80, rate of patients with FSIQ/DQ higher than 80 after therapy; AR, rate of motor function aggravation.

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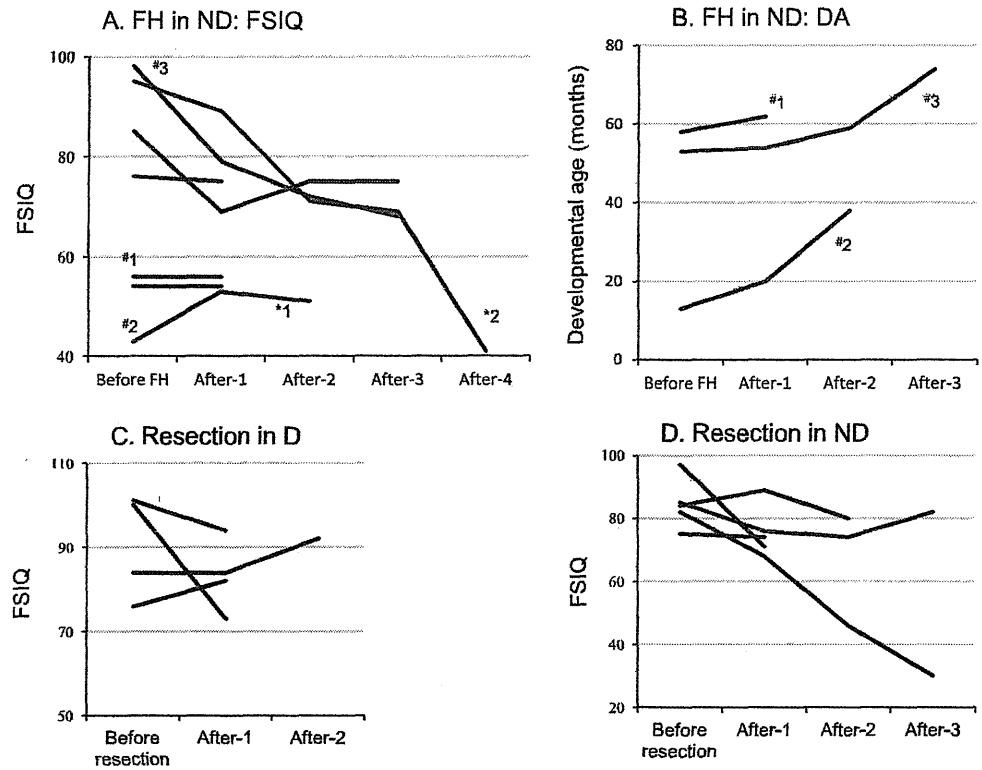

Fig. 4. Evolution of FSIQ/DQ and developmental age after surgical interventions. FSIQ: full scale intelligent quotient; DA: developmental age; after-1, -2, -3 and -4: first, second, third and fourth examinations, respectively, after surgery. A. "FH in ND: FSIQ" shows the evolution of FSIQ/DQ in seven patients treated by FH of the non-dominant hemisphere. *1, relapse of seizures at 3 years after FH; *2, relapse of seizures at 6 years after FH. #1, #2 and #3 in A and B denote the same patients. B. "FH in ND: DA" shows the evolution of DA measured by Tanaka–Binet test in three patients treated by FH. C. "Resection in D" shows the evolution of FSIQ in four patients treated by surgical resection in dominant hemisphere. D. "Resection in ND" shows the evolution of FS.

For precise evaluation of cognitive outcome of surgical intervention, evolution of FSIQ/DQ was studied (Fig. 4). In three patients with FSIQ/DQ higher than 80 before FH of non-dominant hemisphere, FSIQ/DQ decreased gradually after FH to below 80, during periods without seizure relapse (Fig. 4A). On the other hand, in four patients with FSIQ lower than 80 before FH, FSIQ/DQ was maintained at pre-FH levels. In younger patients whose cognitive function was evaluated by developmental age (DA), DA increased slightly after FH, although FSIQ/DQ did not improve (Fig. 4B). Two of four patients treated by surgical resection in dominant hemisphere had FSIQ/DQ higher than 90 before FH, and one showed FSIQ decrease greater than 10 after surgical intervention, without seizure control (Fig. 4C). On the other hand, in two patients with FSIQ lower than 90 before FH, FSIQ was maintained at pre surgical levels. In five patients treated by surgical resection in non-dominant hemisphere, four had FSIQ/DQ higher than 80, two of whom had FSIQ/DQ decrease greater than 10 after surgical intervention, without seizure control (Fig. 4D). On the other hand, in one patient with FSIQ/DQ lower than 80 before surgery, FSIQ/DQ was maintained at the pre surgical level.

R80 after regular pulse therapy was 100% in patients without MRI lesions, 50% in patients with high intensity lesions, and 37% in patients with advanced MRI lesions (Fig. 5A). R80 after tacrolimus therapy was 28% in patients with advanced MRI lesions (Fig. 5B). R80 after regular IVIg therapy was 100% in patients without MRI lesions and patients with high intensity lesions, and 20% in patients with advanced MRI lesions (Fig. 5C).

The relationship between treatment modalities and cognitive outcome is shown in Fig. 6. R80 was 43% by regular pulse therapy followed by tacrolimus therapy, 50% by regular pulse therapy, 33% by regular IVIg therapy, 0% by regular IVIg therapy followed by FH, 33% by regular IVIg therapy followed by surgical resection, 0% by FH of non-dominant hemisphere, 60% by surgical resection, and 33% by surgical resection followed by regular pulse therapy. No patient achieved IQ > 80 by treatments including FH (FH or IVIg followed by FH), but more than 50% of patients achieved IQ > 80 by regular pulse therapy and surgical resection. Mean IQ achieved by IVIg followed by FH (65 ± 15) tended to be higher than that by FH (57 ± 12), and that by surgical resection preceded by IVIg (76 ± 12) tended to be higher than that by surgical resection (65 ± 28)

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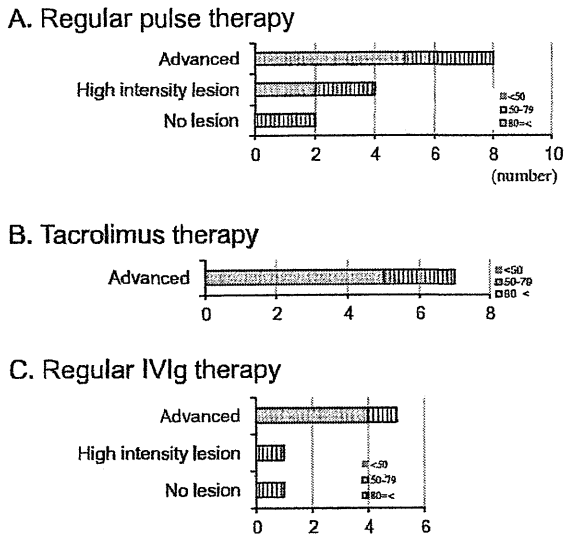

Fig. 5. MRI stage & the last IQ/DQ after immunomodulatory therapies. Horizontal axis shows number of patients with each category of FSIQ/DQ. Advanced, advanced lesion on MRI; High intensity lesion, high intensity MRI lesions; No lesion, without MRI lesion.

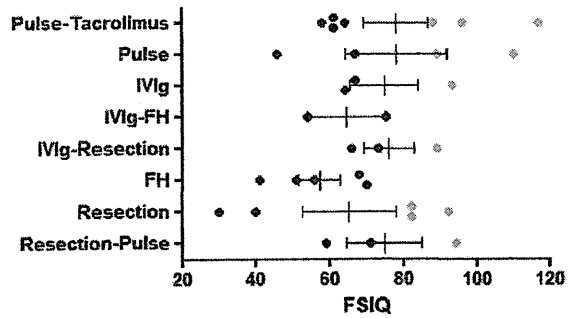

Fig. 6. Treatment modalities and the last FSIQ/DQ. Horizontal axis shows FSIQ. Pulse-Tacrolimus, regular steroid pulse therapy followed by tacrolimus therapy; Pulse, regular pulse therapy; IVIg, regular IVIg therapy; FH, functional hemispherectomy; Resection, surgical resection; Bars show mean ± SE. Purple dots mean data of FSIQ above 80. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

($p > 0.05$). Mean IQ achieved by resection followed by regular pulse therapy (75 ± 18) tended to be higher than that by surgical resection (65 ± 28) ($p > 0.05$).

3.4. Motor outcome

Improvement of motor dysfunction (paresis) was observed in 15% of patients treated by regular IVIg therapy, 10% of patients treated by regular pulse therapy, and 8% of patients treated by tacrolimus therapy (Fig. 7). Aggravation of motor function (progression of motor dysfunction) was observed in 100% of patients

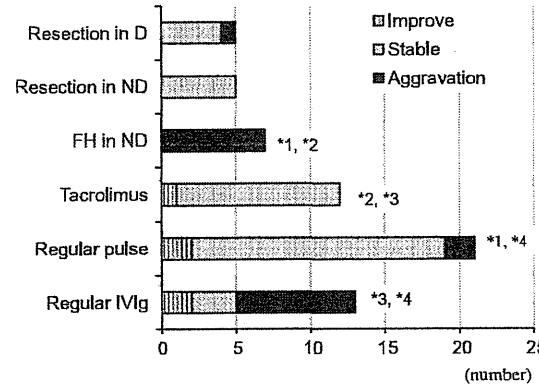

Fig. 7. Motor outcome after surgeries or immunomodulatory therapies. Horizontal axis shows number of patients with each category of cognitive changes. Resection in D, surgical resection in dominant hemisphere; ND, nondominant hemisphere; FH, functional hemispherectomy; Tacrolimus, tacrolimus therapy. Chi-square test for trend detected significant difference in cognitive changes between two therapies marked by (*) (*1, $p < 0.0001$; *2, $p < 0.001$; *3, $p = 0.0314$; *4, $p = 0.314$).

treated by FH, 62% of patients treated by regular IVIg, 20% of patients treated by surgical resection in dominant hemisphere, and 10% of patients treated by regular pulse therapy. Motor outcome by regular pulse therapy was significantly better than that by FH of non-dominant hemisphere and regular IVIg therapy. Motor outcome by tacrolimus therapy was significantly better than that by FH of non-dominant hemisphere and regular IVIg therapy.

3.5. Discontinuation of immunomodulatory therapies and adverse events

Regular IVIg therapy was discontinued in 100% (13/13) of the patients (Table 2), and treatment was switched to regular steroid pulse therapy in two patients, tacrolimus therapy in three, surgical intervention in three, and AEDs only in five. Significant adverse events were not observed. The reasons for discontinuation included aggravation of seizures, aggravation of motor dysfunction, and medical costs.

Regular pulse therapy was discontinued in 62% (13/21) of the patients, and treatment was switched to regular IVIg therapy in one patient, tacrolimus therapy in seven, surgical intervention in one, and AEDs only in four. Significant adverse events were not observed. The reason for discontinuation was disturbance of quality of life due to regular hospitalization for longer periods.

Tacrolimus therapy was discontinued in 17% (2/12) of the patients, and treatment was switched to regular pulse therapy in one patient and surgical intervention in one. Significant adverse events were not observed. The reasons for discontinuation included aggravation of seizures and aggravation of motor dysfunction.

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4. Discussion

FH has been the major treatment for RS. In the current treatment strategy for RS, the indication of FH is considered as soon as RS is diagnosed [2]. From the viewpoint of seizure outcome, FH is the only treatment to achieve complete seizure control in RS, but the seizure-free rate of FH is not 100%; the rate was reported to be 62.5–85% in the literature [2]. From the viewpoint of cognitive outcome, we found that all patients with FSIQ/DQ higher than 80 before FH experienced reduction in IQ/DQ to levels below 80 after FH, but R80 after immunomodulatory therapy was 29–50%. Regarding motor outcome, FH inevitably results in deterioration of motor function. Although FH is an important beneficial treatment, many issues await solutions. We need to establish innovative treatment strategies that can improve seizure outcome as well as preserve cognitive and motor functions. Evidence of the efficacy of immunomodulatory treatments has accumulated, in this study we compared the outcomes among surgical intervention, regular IVIg therapy, regular pulse therapy and tacrolimus therapy, mainly in patients with pediatric onset RS. Based on the results of analyses, we attempted to propose innovative treatment strategies.

Among the various immunomodulatory therapies and surgical interventions, regular IVIg therapy showed relatively poor seizure outcome, average cognitive outcome, and poor motor outcome (Table 2). Regular IVIg therapy was discontinued, mainly because of aggravation of seizures, and/or deterioration of motor functions. These data suggested that regular IVIg therapy in patients with pediatric onset had disadvantages in seizure control and preserving motor functions. On the other hand, favorable responses in adult cases have led to the proposal of IVIg as first-line treatment especially in late onset cases [9,10]. Further studies on the efficacy of IVIg considering the age at treatment are needed.

Regular steroid pulse therapy showed relatively good seizure outcome, good cognitive outcome, and good motor outcome (Table 2). Regular pulse therapy had the highest response rate for seizure outcome among the immunomodulatory therapies, and this therapy reduces frequent intractable seizures in the acute stage. Regular steroid pulse therapy also had the best cognitive outcome among all treatments other than surgical resection in dominant hemisphere, although this treatment does not achieve complete seizure control. Motor outcome was good and deterioration of motor function was infrequent. Although short-term intravenous bolus administration of methylprednisolone has been reported to be effective in blocking status epilepticus [10,11], the efficacy of regular pulse therapy administered for several months has not been reported. Cognitive outcome of regular pulse therapy seemed to be better in earlier stages, because R80 was higher in patients without MRI lesions, compared to patients with advanced MRI lesions. These data suggest that regular pulse therapy may contribute to seizure control and cognitive preservation in early-stage RS. However, the treatment was discontinued in 62% of patients, mainly due to frequent hospitalization which disturbs school life. The therapy was replaced by tacrolimus therapy in seven of 13 patients. These data suggest that quality of life has to be considered in planning treatment strategies for RS patients.

Tacrolimus therapy showed moderately good seizure control, relatively good cognitive outcome, and very good motor outcome (Table 2). In a previous study, tacrolimus-treated patients had superior outcome in neurological and cognitive functions, but no better seizure outcome compared to untreated patients [12]. These data suggest that tacrolimus therapy can maintain cognitive and motor function, in spite of relatively inferior seizure control.

The diagnostic criteria of RS include clinical symptoms, EEG findings, and MRI characteristics suggesting unilateral cortical deficit [2]. Therefore, many patients have already more or less permanent disturbance of motor and cognitive functions when RS is confirmed, and FH is accepted mainly by patients with non-dominant hemisphere involvement. However, FH has the issues of not achieving 100% SFR and poor cognitive outcome, especially in patients with higher IQ. To improve the outcome of RS, we suggest a new treatment strategy using early immunomodulatory therapies (Fig. 8). Recent immunological studies in RS revealed a pivotal role of cytotoxic T cells, and proposed biomarkers such as CSF levels of granzyme B and IFNγ in early-stage RS without permanent neurological involvement [4,5]. Within one year of seizure onset, 60% of patients had high intensity lesions (HIL) on MRI [8]. Therefore HIL may be one of the early markers suggesting RS. While granzyme B and IFNγ in CSF

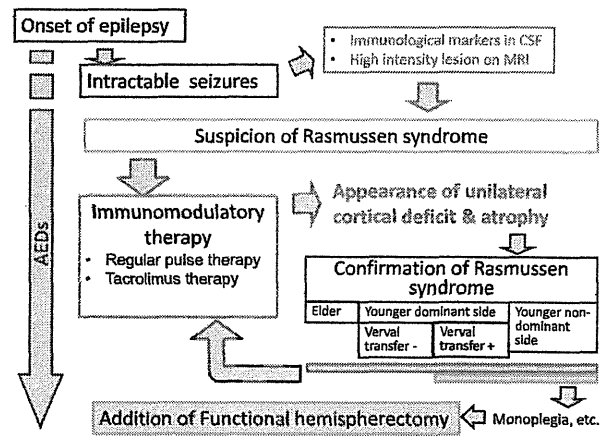

Fig. 8. New treatment strategy for Rasmussen syndrome with pediatric onset.

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are early stage markers, HIL may also contribute to an early suspected diagnosis of RS and indicate the timing of starting immunomodulatory therapies before the appearance of unilateral cortical deficit. Because regular pulse therapy yields superior seizure outcome as well as better cognitive outcome in early stage before the appearance of MRI lesions than in later stage, we recommend regular pulse therapy as first-line immunomodulatory therapy in patients with suspected RS. After several to 12 months of regular pulse therapy when seizures become stable, switching to tacrolimus therapy is recommended so that therapy can be conducted mainly on an out-patient basis. In the course of immunomodulatory therapies, appearance of unilateral cortical deficits necessitates prompt addition of FH. When the neurological deficits manifested are equivalent to those that would inevitably result from FH, then FH is indicated mainly in patients with disease involving the non-dominant hemisphere. In patients with disease involving the dominant hemisphere, FH can be considered if verbal transfer is possible.

5. Conclusion

For the improvement of outcome of RS, there seems to be a place for immunomodulatory treatments in pediatric patients, and the treatments are recommended in the early stages, preferably before any motor or cognitive dysfunction and among no MRI lesions.

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.

Disclosure of conflicts of interest

All authors have no conflict of interest to disclose.

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Case report

Anti-*N*-methyl D-aspartate-type glutamate receptor antibody-positive limbic encephalitis in a patient with multiple sclerosis

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1. Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system. Although it often coexists with other autoimmune diseases, its association with anti-*N*-methyl D-aspartate receptor (NMDAR) antibody-positive encephalitis, which is characterized by fulminant prominent neuropsychiatric manifestations, seizures, dyskinesias and autonomic instability [1], is rare. In this study, we describe the case of a Japanese female MS patient who developed with anti-NMDA type glutamate receptor (GluR) antibody-positive limbic encephalitis. The simultaneous manifestation of both diseases has never been reported to the best of our knowledge.

2. Case report

A 33-year-old Japanese woman developed left optic neuritis (ON) at the age of 30. She experienced some demyelinating inflammatory episodes, including the development of 2 left optic nerve lesions, 3 different spinal cord lesions and 1 brainstem lesion between the ages of 31 and 33 years. Intravenous high-dose methylprednisolone pulse (IVMP) therapy was effective for treating the acute inflammatory episodes. The patient's relapses, in addition to the observation of clinical lesions on magnetic resonance images (MRI), led to the diagnosis of MS according to 2005 McDonald's criteria.

At age 33, the patient developed fatigue and fever. Three weeks after manifestation of prodromal symptoms, she developed epileptic seizures and lost consciousness following psychobehavioral symptoms; she was then admitted to our hospital for further investigation. Upon admission, she was conscious and responsive, but presented with mild cognitive deficits (verbal and performance IQ of 88 and 78, respectively, as determined by the Wechsler Adult Intelligence Scale-R), manic, persecution complex and over-interfering to others. Neurological examination revealed loss of visual acuity, left abductor muscle weakness, mild left hemiparesis and left hypoesthesia below the C4 and T7 dermatomal areas. Cerebrospinal fluid (CSF) parameters on admission were as follows: cell count, 3 cells/mm^3; protein level, 35 mg/dl; immunoglobulin G index, 0.700; positive for oligoclonal bands and no evidence of any active viral infections. Laboratory findings were unremarkable and negative for antinuclear, anti-SS-A/B, anti-thyroid peroxidase or anti-aquaporin-4 antibodies. Brain MRI demonstrated hyperintensity of the bilateral medial temporal lobes, some periventricular ovoids on FLAIR images and multifocal white matter lesions with gadolinium enhancement (Fig. 1). Spinal cord MRI revealed a solitary C2 lesion. Epileptic discharges were not noted on some times of electroencephalograms. She was diagnosed with limbic encephalitis instead of an MS exacerbation and was treated with IVMP (1 g/day for 3 days) followed by oral prednisolone (30 mg/day). The aetiology of the patient's psychobehavioral symptoms and seizures was further examined, and it was determined that antibodies against the GluRε2 subunit were in her CSF, but not the serum. Systemic computed tomography/MRI/ultrasonography did not detect any tumours. Although she presented with epileptic seizures and required ventilatory support 2 months after admission, her symptoms were ceased not immediately but slowly. She was discharged without neurological deficits, psychobehavioral symptoms or epileptic seizures 6 months later.

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