EARLY PREDICTORS AND PREVENTION FOR POST-STROKE EPILEPSY: CHANGES IN NEUROTRANSMITTER LEVELS

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Abstract

Background: The purpose of this study was to identify predictors and preventative treatments for post-stroke epilepsy (PSE). Methodology: Eighty-four patients who had suffered a cerebrovascular insult (within 72 hours) were recruited and divided into two groups: an EP group (patients with seizures after stroke) and a NEP group (patients without seizures after stroke). The NEP group was then subdivided into three groups: a control group, a GABA (γ-aminobutiric acid) group (received GABA orally), and a CCB group (received calcium channel blocker nimodipine orally). Patient groups were compared by gender, age, past medical history, stroke type, number of lesions, and position and stroke severity (using Scandinavian stroke scale, SSS). Forearm venous blood was sampled, and high performance liquid chromatography (HPLC) was used to measure plasma levels of neurotransmitters and Ca^{2+}. Patients then received 14 days of drug intervention. One month after drug withdrawal, GABA, glutamate (Glu) and Ca^{2+} concentrations in plasma were measured again. Results: The number of previous strokes, size of infarction, presence of multiple lesions, localization to the cortex, and SSS were statistically significant between the two groups ($P < 0.05$). In the EP group, the Glu concentration was greater and the Ca^{2+} concentration was lower than in the NEP group ($P < 0.05$). The results obtained after 1 month of therapy showed a reduction in Glu levels and an increase in GABA levels in the GABA group relative to the control NEP group ($P < 0.05$), while the CCB group showed a decrease in the concentration of Glu and an increase in the concentrations of GABA and Ca^{2+} relative to the NEP control group ($P < 0.05$). Conclusions: We identified susceptibility factors for PSE and demonstrated that GABA and calcium antagonists may have a therapeutic use in the early prevention of PSE.

Introduction

Stroke is a major risk factor for the development of epilepsy, especially in the elderly [1]. The incidence of seizures was found to be 10.6% in 265 patients with intracerebral hemorrhage and 8.6% among 1,632 patients with ischemic stroke [2]. Several seizure predictors have been studied including: stroke subtype, size, location, severity of the vascular lesion, and the presence of previous seizures [3-5]. However, there is little data available regarding both early warning signs and preventative therapies for post-stroke epilepsy (PSE).

Based on the time of onset following the initial cerebral ischemic event, seizures are traditionally divided into two types: early and late [2]. In general, the former refers to the onset of seizures within 2 weeks after stroke, and the latter refers to the onset of seizures after 2 weeks following stroke [6-8]. In stroke patients, 35% of patients with early onset seizures and 90% with late onset seizures developed epilepsy [9]. In most patients with early-onset seizures, the seizures began within 1-2 days after the infarction [10].

The pathophysiology of epilepsy after stroke is complex. Neurotransmitter amino acids play an important role in the pathogenesis and development of epilepsy. The excitatory neurotransmitter glutamate (Glu) and the inhibitory neurotransmitter γ-aminobutyric acid (GABA) have a close relationship, where a reduction in GABA is associated with an increase in Glu. Increased concentrations of Glu cause excitotoxicity, disturbance of electrolyte balance, destruction of phospholipid membranes, and secretion of free fatty acids. These changes have been documented in the penumbral areas in the acute post-stroke phase [11-13]. In addition, the accumulation of intracellular calcium and sodium results in depolarization of the transmembrane potential, and this, taken together with other calcium-mediated effects, can lower the seizure threshold [2]. Based on these aspects of amino acid neurotransmitters and ion levels, we performed an intervention study.

In this study, we aimed to identify early predictors of PSE by measuring changes in blood levels of GABA, Glu, and calcium (Ca^{2+}) in order to develop preventative therapeutic strategies for PSE.

Methods

The study was performed with the approval of the ethics committee of the First hospital of Jilin University. Written informed consent was obtained from all patients or guardians of

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patients participating in the study.

Patients with acute cerebrovascular disease hospitalized in our hospital from May 2011 to August 2012 were enrolled in this study. In all cases, patients without prior seizures, diagnostically confirmed by head CT or MRI, were sent to the hospital within 72 hours, which was in accordance with the diagnostic criteria passed in the fourth National Cerebrovascular Disease Conference in 1996. Patients in the epilepsy group (EP, seizures after stroke) were identified based on the definitions of epilepsy in the International League Against Epilepsy (ILAE, 1989). Patients with additional territorial infarcts were excluded from the study. Patients diagnosed as transient ischemic attack (TIA), subarachnoid hemorrhage (SAH), or who had had prior surgical treatment were also excluded. All patients used statins (atorvastatin, 20 mg/day).

In order to identify predictors of PSE, patients were divided into two separate groups: the EP group and the NEP (no epilepsy after stroke) group. Patients in the EP group received antiepileptic drugs, as needed, to treat epilepsy. To identify preventative treatments for PSE, the NEP group was further divided into three subgroups: control group, GABA intervention group (GABA group), and calcium channel blocker intervention group (CCB group).

Patient groups were compared on the following parameters: gender, age, past medical history (stroke, hypertension, diabetes, coronary heart disease, atrial fibrillation, and smoking), stroke type, number of lesions, and location and stroke severity (Scandinavian stroke scale, SSS). Forearm venous blood was sampled after fasting, and high performance liquid chromatography (HPLC) was used to measure plasma levels of GABA, Glu, and Ca^{2+}. Patients in the NEP group then received a drug of intervention for 14 days. Patients in the GABA group received 100 mg GABA orally three times a day, whereas patients in the CCB group received 30 mg nimodipine orally, three times a day. One month after drug withdrawal, GABA, Glu, and Ca^{2+} levels in plasma were measured again.

All data were analyzed with SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The normality test was used to determine if data were normally distributed. For non-normally distributed data, median values are reported and the Wilcoxon rank-sum test was utilized. For paired t test analysis, it was supposed that variances were not necessarily equal. Values for the concentration of plasma amino acids and Ca^{2+} were expressed as mean ± standard deviation (SD). Ratios were compared by Chi-square test. The statistical significance was set at an α level of 0.05.

Results

A total of 84 patients (41 women, 43 men; age 38-80 years) were enrolled in this study and divided into two groups: EP group (n = 20) and NEP group (n = 64). The NEP group was further divided into control group (n = 17), GABA group (n = 26), and CCB group (n = 21).

After taking GABA and nimodipine, three patients had dry stool, four had transient dizziness, and two had nausea. Adverse reactions were mild, with the longest duration being 3 days.

There was no significant difference in sex ratio or average age between the EP and NEP groups. In the EP group, the incidence of previous stroke history was significantly greater than in the other group (P < 0.05). Constituent ratio of the infarction in the EP group was significantly higher than the NEP group (P < 0.05). Relative to the NEP group, a greater proportion of patients in the EP group had multiple lesions (P < 0.05), and these lesions were more likely to be localized in the cortex (P < 0.001). We also found that the more severe the stroke (SSS < 30), the greater the number of seizures the patients had (P < 0.05) (data summarized in Table 1).

When we compared EP group to NEP group, we found that Glu concentration increased,

Table 1. General characteristics of the patient population (n = 84). EP, epilepsy after stroke; NEP, no epilepsy after stroke; *Scandinavian stroke scale.

	EP group		NEP group		χ^2	
	n	(%)	n	(%)		P
Past medical history						
Stroke	14	70.00	26	40.63	5.271	0.022
Hypertension	11	55.00	35	54.69	0.001	0.980
Diabetes	9	45.00	19	29.69	1.608	0.205
Coronary heart disease	6	30.00	15	23.44	0.350	0.554
Atrial fibrillation	5	25.00	17	26.56	0.019	0.890
Smoking	12	60.00	28	43.75	1.613	0.264
Stroke-type						
Infarction	15	75.00	30	46.88	4.846	0.028
Hemorrhage	5	25.00	34	53.12		
Number of lesions						
Single	6	30.00	37	57.81	4.718	0.03
Multifocal	14	70.00	27	42.19		
Position						
Cortical	13	65.00	14	21.86	12.993	< 0.001
Subcortical	7	35.00	50	87.12		
Stroke severity (SSS* score)						
<30	9	45.00	13	20.31	4.804	0.028
>30	11	55.00	51	79.69		

and Ca^{2+} concentration decreased ($P < 0.05$). However, in the GABA group, Glu concentration decreased ($P < 0.05$) and GABA concentration increased with the intervention ($P < 0.05$) relative to the control group. There was no significant difference in Ca^{2+} concentration between control and GABA groups ($P > 0.05$). Therefore, oral GABA increased GABA and decreased Glu levels, which could play an important role in the inhibition of epileptic seizures. In the CCB group, there was no significant change in GABA or Glu concentrations ($P > 0.05$), but the Ca^{2+} concentration was significantly increased with this intervention ($P < 0.05$) relative to the control group. Therefore, oral nimodipine increased Ca^{2+} concentration in plasma of patients with acute cerebrovascular disease, indicating that inhibition of Ca^{2+} flow by nimodipine could prevent the occurrence of PSE (data summarized in Table 2).

Discussion

In this study, we evaluated the reliability of previously proposed predictors of seizures following stroke. We found that ischemic stroke, cortical location, and a severe stroke score (SSS < 30) were possible predictors for seizure development.

The age of the patients ranged from 45 to 79 years old in the EP group, and we found no evidence that age is a significant predictor for PSE. This is consistent with a report by Paolucci et al. [14] who found that the risk of seizures in younger patients relative to older ones was small and not statistically significant [5].

Previous studies have shown that the number of lesions, their location, and severity are risk factors for the development of PSE [7, 10, 15-17]. Multivariate analysis of data from seizures occurring after stroke identified a cortical location as a significant risk factor [2]. In addition, both population-based [7] and prospective multicenter studies [2] reported that stroke severity was independently linked with the development of seizures after ischemic stroke [4]. Indeed, it was found that severe strokes, defined as SSS score < 30 at hospital admission, were a significant predictor for the development of PSE [5].

Unlike previous studies [2, 15, 18, 19], we did not determine if hemorrhagic stroke was a significant predictor of seizures. There is an association between intracerebral hemorrhage and early seizures, especially for subarachnoid hemorrhage [20-22]. The mechanism of hemorrhage induced seizure initiation remains unclear. Hemosiderin, a product of blood metabolism, may cause focal cerebral irritation by promoting iron deposition in the cerebral cortex leading to seizures [23]. Perhaps the sequestration of hemosiderin in cortical neurons plays a role in seizures following traumatic brain injury as well [24]. In this study, we only evaluated whether ischemic stroke was a significant predictor for PSE. Given the small sample size, short follow-up period, and economic limitations, the role of subarachnoid hemorrhage was not examined.

Statins are commonly used to lower cholesterol levels and are considered first-line agents in the primary and secondary prevention of cardiovascular and cerebrovascular diseases. There is mounting evidence that statins play a protective role in acute stroke, Alzheimer's disease, Parkinson's disease, multiple sclerosis, and epilepsy [25-28]. Etminan et al. reported that statins can reduce the risk of hospitalization for epilepsy in the elderly [29], but the mechanism of action was unclear. Therefore, all patients in our study received atorvastatin (20 mg/day) in order to reduce the potential variation due to statins.

Acute ischemia leads to increased extracellular concentrations of glutamate, an excitatory neurotransmitter that has been associated with secondary neuronal injury [10, 30]. Glutamate excitotoxicity is a well-established mechanism of cell death in an experimental stroke model, and brain damage causes widespread neuronal depolarization, leading to massive simultaneous release of Glu and GABA [31]. Anti-glutamatergic drugs may actually impair recovery after ischemia and other forms of brain injury by affecting neuronal processes apart from the treatment of seizures. Exogenous oral GABA normally cannot cross the blood brain barrier. However, under conditions where there are lesions or other blood brain barrier disorders, GABA can enter the brain. This mechanism may account for the effectiveness of orally administered GABA in our study. During acute ischemic injury, accumulation of intracellular Ca^{2+} and sodium causes depolarization of the transmembrane potential and activation of signaling pathways downstream of Ca^{2+}, as well

Table 2. Plasma concentrations of GABA, Glu, and Ca^{2+}. Glu, glutamate; EP, epilepsy after stroke; NEP, no epilepsy after stroke; CCB, calcium channel blocker.

	EP group (n = 20)	NEP group (n = 64)		
		Control group (n = 17)	GABA group (n = 26)	CCB group (n = 21)
Plasma concentrations of Glu				
Within 72 hours (mg/L)	29.42 ± 2.40	19.69 ± 5.28	30.36 ± 4.45	27.05 ± 5.10
1 month after onset (mg/L)	-	16.82 ± 3.87	21.72 ± 6.33	25.23 ± 2.60
Plasma concentrations of GABA				
Within 72 hours (mg/L)	1.81 (1.79, 1.82)	3.20 ± 1.19	2.03 ± 0.47	1.51 ± 0.65
1 month after onset (mg/L)	-	1.71 ± 0.62	2.56 ± 0.37	1.56 ± 0.75
Plasma concentrations of Ca^{2+}				
Within 72 hours (mg/L)	2.16 ± 0.12	2.22 ± 0.07	2.25 ± 0.04	2.18 ± 0.06
1 month after onset (mg/L)	-	2.12 ± 0.06	2.27 ± 0.04	2.29 ± 0.07

as shifts in ionic potential. Ca^{2+} channel blockers may lower the seizure threshold [10, 32]. In the hippocampus of epileptic rats, the permeability and intracellular flow of Ca^{2+} was increased and the L-type calcium channel blocker nifedipine blocked this Ca^{2+} influx [33]. Many animal and clinical studies have confirmed the anti-epileptic effect of intravenously administered nimodipine, another L-type calcium channel blocker [34]. In this study, administration of nimodipine resulted in elevated blood Ca^{2+} by inhibiting Ca^{2+} influx and preventing onset of epilepsy.

Further studies with long-term follow-up are necessary to investigate whether seizures in stroke patients can be prevented through the use of these treatments. Seizures increase Glu concentration and decrease Ca^{2+} concentration in the plasma of acute cerebrovascular accident patients. A history of stroke and stroke involving the cortex with multiple lesions and a greater stroke severity (SSS < 30) are all independent susceptibility factors for PSE. Orally administered GABA increased the concentration of GABA and reduces the concentration of Glu. Oral calcium channel antagonists can inhibit the flow of Ca^{2+},

which is important for early prevention and treatment. Taken together, our findings identify important risk factors for the development of PSE and two possible therapeutic options for the pharmacological treatment of PSE.

Acknowledgment

References

[1] Li X., Breteler M., Bruyne M.C., Meinardi H., Hauser W.A., Hofman A., Vascular determinants of epilepsy: the Rotterdam Study, Epilepsia, 1997, 38, 1216-1220

[2] Bladin C.F., Alexandrov A.V., Bellavance A., Bornstein N., Chambers B., Coté R., et al., Seizures after stroke: a prospective multicenter study, Arch. Neurol., 2000, 57, 1617-1622

[3] Beghi E., D'Alessandro R., Beretta S., Consoli D., Crespi V., Delaj L., et al., Incidence and predictors of acute symptomatic seizures after stroke, Neurology, 2011, 77, 1785-1793

[4] Camilo O., Goldstein L.B., Seizures and epilepsy after ischemic stroke, Stroke, 2004, 35, 1769-1775

[5] Lossius M.I., Rønning O.M., Slapø G.D., Mowinckel P., Gjerstad L., Poststroke epilepsy: occurrence and predictors - a long-term prospective controlled study (Akershus Stroke Study), Epilepsia, 2005, 46, 1246-1251

[6] Olafsson E., Gudmundsson G., Hauser W.A., Risk of epilepsy in long-term survivors of surgery for aneurysmal subarachnoid hemorrhage: a population-based study in Iceland, Epilepsia, 2000, 41,1201-1205

[7] Reith J., Jørgensen H.S., Nakayama H., Raaschou H.O., Olsen T.S., Seizures in acute stroke: predictors and prognostic significance. The Copenhagen Stroke Study, Stroke, 1997, 28, 1585-1589

[8] Sung C.-Y., Chu N.-S., Epileptic seizures in intracerebral haemorrhage, J. Neurol., Neurosurg. Psychiatry, 1989, 52, 1273-1276

[9] Sung C.-Y., Chu N.-S., Epileptic seizures in thrombotic stroke, J. Neurol., 1990, 237, 166-170

[10] Silverman I.E., Restrepo L., Mathews G.C., Poststroke seizures, Arch. Neurol., 2002, 59, 195-201

[11] Kessler K.R., Schnitzler A., Classen J., Benecke R., Reduced inhibition within primary motor cortex in patients with poststroke focal motor seizures, Neurology, 2002, 59, 1028-1033

[12] Sun D.A., Sombati S., Blair R.E., DeLorenzo R.J., Calcium-dependent epileptogenesis in an in vitro model of stroke-induced "epilepsy", Epilepsia, 2002, 43, 1296-1305

[13] Sun D.A., Sombati S., DeLorenzo R.J., Glutamate injury-induced epileptogenesis in hippocampal neurons an in vitro model of stroke-induced "epilepsy", Stroke, 2001, 32, 2344-2350

[14] Paolucci S., Silvestri G., Lubich S., Pratesi L., Traballesi M., Gigli G.L., Poststroke late seizures and their role in rehabilitation of inpatients, Epilepsia, 1997, 38, 266-270

[15] Alberti A., Paciaroni M., Caso V., Venti M., Palmerini F., Agnelli G., Early seizures in patients with acute stroke: frequency, predictive factors, and effect on clinical outcome, Vasc. Health Risk Manag., 2008, 4, 715-720

[16] Misirli H., Özge A., Somay G., Erdoğan N., Erkal H., Erenoğlu N., Seizure development after stroke, Int. J. Clin. Pract., 2006, 60, 1536-1541

[17] Myint P., Staufenberg E., Sabanathan K., Post-stroke seizure and post-stroke epilepsy, Postgrad. Med. J., 2006, 82, 568-572

[18] Lamy C., Domigo V., Semah F., Arquizan C., Trystram D., Coste J., et al., Early and late seizures after cryptogenic ischemic stroke in young adults, Neurology, 2003, 60, 400-404

[19] Szaflarski J.P., Rackley A.Y., Kleindorfer D.O., Khoury J., Woo D., Miller R., et al., Incidence of seizures in the acute phase of stroke: a population-based study, Epilepsia, 2008, 49, 974-981

[20] Burn J., Dennis M., Bamford J., Sandercock P., Wade D., Warlow C., Epileptic seizures after a first stroke: the Oxfordshire Community Stroke Project, Brit. Med. J., 1997, 315, 1582-1587

[21] Hart R.G., Byer J.A., Slaughter J.R., Hewett J.E., Easton D.J., Occurrence and implications of seizures in subarachnoid hemorrhage due to ruptured intracranial aneurysms, Neurosurgery, 1981, 8, 417-421

[22] Sundaram M., Chow F., Seizures associated with spontaneous subarachnoid hemorrhage, Can. J. Neurol. Sci., 1986, 13, 229-231

[23] Buchkremer-Ratzmann I., August M., Hagemann G., Witte O.W., Epileptiform discharges to extracellular stimuli in rat neocortical slices after photothrombotic infarction, J. Neurol. Sci., 1998, 156, 133-137

[24] Küçükkaya B., Aker R., Yüksel M., Onat F., Yalçin A.S., Low dose MK-801 protects against iron-induced oxidative changes in a rat model of focal epilepsy, Brain Res., 1998, 788, 133-136

[25] Banach M., Czuczwar S.J., Borowicz K.K., Statins – are they anticonvulsant?, Pharmacol. Rep., 2014, 66, 521-528

[26] Reiss A.B., Wirkowski E., Statins in neurological disorders: mechanisms and therapeutic value, ScientificWorldJournal, 2009, 9, 1242-1259

[27] Stępień K., Tomaszewski M., Czuczwar S.J., Neuroprotective properties of statins, Pharmacol. Rep., 2005, 57, 561-569

[28] van der Most P.J., Dolga A.M., Nijholt I.M., Luiten P.G., Eisel U.L., Statins: mechanisms of neuroprotection, Prog. Neurobiol., 2009, 88, 64-75

[29] Etminan M., Samii A., Brophy J.M., Statin use and risk of epilepsy: a nested case-control study, Neurology, 2010, 75, 1496-1500

[30] Luhmann H.J., Ischemia and lesion induced imbalances in cortical function, Prog. Neurobiol., 1996, 48, 131-166

[31] Willmore L.J., Post-traumatic seizures, Neurol. Clin., 1993, 11, 823-834

[32] Forsgren L., Bucht G., Eriksson S., Bergmark L., Incidence and clinical characterization of unprovoked seizures in adults: a prospective population-based study, Epilepsia, 1996, 37, 224-229

[33] Hayes R.L., Jenkins L.W., Lyeth B.G., Neurotransmitter-mediated mechanisms of traumatic brain injury: acetylcholine and excitatory amino acids, J. Neurotrauma, 1992, 9, S173-187

[34] Amano T., Amano H., Matsubayashi H., Ishihara K., Serikawa T., Sasa M., Enhanced Ca^{2+} influx with mossy fiber stimulation in hippocampal CA3 neurons of spontaneously epileptic rats, Brain Res., 2001, 910, 199-203