Comparative Study of Preventive and Therapeutic Effects of IEM-1966 and Memantine in Rats with Experimental Allergic Encephalomyelitis

I. N. Abdurasulova, S. E. Serdyuk, and V. E. Gmiro

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We compared preventive and therapeutic effects of memantine, a selective blocker of NMDA-receptors, and IEM-1966, a blocker of both NMDA- and GluR1 AMPA-receptors, on the model of acute experimental allergic encephalomyelitis. Memantine in high doses prevented the development of experimental allergic encephalomyelitis only in 10% rats, slightly (by 1.4-1.5 times) moderated the neurological disturbances, and shortened the duration of the disease. In far lower doses, IEM-1966 prevented the development of experimental allergic encephalomyelitis in 50% rats, while in the affected rats it decreased the severity of neurological disturbances and duration of the disease by 3-4 times. When applied during the clinical phase of the disease, IEM-1966 decreased the severity of neurological disturbances and duration of the disease by 2.0-2.5 times predominantly in rats with mild and moderate course of experimental allergic encephalomyelitis.

Key Words: *multiple sclerosis; encephalomyelitis; NMDA; AMPA; memantine*

In humans, multiple sclerosis is a severe autoimmune demyelinating disease rapidly leading to disability [8]. Experimental allergic encephalomyelitis (EAE) in animals is a widely-used model of multiple sclerosis, because both diseases have similar pathogenetic mechanisms and clinical manifestations [8,11,12].

In the inflammatory and autoimmune cascade in CNS, the triggering role is played to proinflammatory cytokines formed by astroglial and microglial cells, as well as by macrophages and T-cells migrating across the damaged blood-brain barrier (BBB) [6,9-11,13]. Activation of proinflammatory cytokines (IL-1β, TNF-α) triggers enhanced production of glutamate, which exerts excitotoxic action on neurons and oligodendrocytes due to hyperactivation of NMDA- and AMPA-receptors in neuronal soma and AMPA-receptors in axons and oligodendrocytes [1,4-6,9-11,14,15]. In addition, NMDA-receptors are expressed on cells forming BBB and participating in the regulation of its permeability [6, 9-11]. Thus, moderation of activity of glutamate receptors can be a novel strategy in the therapy of multiple sclerosis.

This approach with selective blockers of NMDA- and AMPA-type receptors was successfully used on rat model of EAE [1,5,9,11,13]. IEM-1966 synthesized by us blocks glutamate receptors of NMDA- and GluR1 AMPA-types [3]. We hypothesize that this agent possesses more pronounced preventive and therapeutic action in rats with EAE in comparison with selective NMDA- and AMPA-blockers, because it affects different pathogenic elements of EAE. Blockade of NMDA-receptors on BBB-forming cells would decrease permeability of this barrier. Complementary block of AMPA-receptors on

S. V. Anichkov Department of Neuropharmacology, I. P. Pavlov Department of Physiology, State Research Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg

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oligodendrocytes would prevent the death of myelin-producing cells. Blockade of NMDA- and AMPA-receptors on neurons will weaken excitotoxicity.

The aim of the present work was to compare the preventive and therapeutic effects of memantine and IEM-1966 on the model of acute EAE in rats.

MATERIALS AND METHODS

EAE was induced in 3-month female Wistar rats weighing 170-190 g (n=59) by a single subcutaneous inoculation of encephalitis-producing mixture consisting of 100 mg homogenate of the homologous spinal cord dissolved in 0.2 ml complete Freund's adjuvant (the content of killed mycobacteria 5 mg/ml) and 0.2 ml physiological saline (per animal). The mixture (0.4 ml) was injected into the tail base under light ether narcosis [1].

Memantine (3,5-dimethyl-1-adamantan hydrochloride, Sigma) in doses of 10 mg/kg (n=9) or 20 mg/kg (n=8) and IEM-1966 [1-amino-4-(1-adamantanmethylamino)butane dihydrobromide] in doses of 0.1 mg/kg (n=8) and 0.3 mg/kg (n=8) were injected daily intraperitoneally in a volume of 1 ml on day 2 through day 16 after induction of EAE (preventive course). In some experiments, IEM-1966 (0.3 mg/kg) was injected on day 8 through day 18 after EAE induction (n=8, therapeutic course). Control rats with EAE (n=18) received physiological saline according to the same scheme.

The rats were weighted, and their neurological state was assessed every day over 30 days (the mean duration of EAE). The onset of EAE after inoculation, its duration, and the degree of neurological symptoms (assessed by clinical index CI, [1]) were analyzed. CI was determined according to the degree and generalization of neurological symptoms: weakness, paresis, or paralysis of muscle on one extremity corresponded to 0.5, 1, or 1.5 points, respectively. When two or more extremities were involved, their scores were summed. The absence of visible disturbances and lethal outcome corresponded to 0 and 6 points, respectively. The course of EAE was termed as mild (CI=0.5-1.5), moderate (CI=2.5-2.5), severe (CI=3.0-4.0), and very severe (CI=4.5-6.0).

The preventive effect of the examined substances on EAE in each group was evaluated by the latency, percent of rats with EAE and severe EAE, mean CI at the peak of EAE, and mean duration of the disease. All parameters were compared with control values.

CI and cumulative CI were analyzed using non-parametric Mann—Whitney U test, latency and duration of clinical phase of EAE were analyzed using Student's t test, and the difference in the percent of rats with EAE and severe EAE by Fisher test.

RESULTS

Inoculation of the encephalitis-producing mixture induced neurological abnormalities in 94.5% control rats injected with physiological saline from post-inoculation day 2 through day 16 (Table 1) and in 100% control rats injected from day 8 through day 18 (Table 2). In these rats, histological signs of EAE (perivascular infiltrates and meningeal inflammation combined with demyelination foci in the cerebellum, brainstem, and in all subdivision of the spinal cord) were detected on the frozen sections of the brain [2].

TABLE 1. Effect of Course Treatment with Memantine and IEM-1966 (on Days Days 2-16 after EAE Induction, Combined Scheme) on Latency, Duration, and Severity of EAE in Female Wistar Rats

Group	Percentage of rats with EAE		EAE latency, days		Mean CI at the peak of EAE, score	Mean cumulative CI, score	EAE duration, days
	total	severe course	mild course	severe course			
Control	94.5	63	12.0±1.5	8.9±1.1	2.2	25.6	15.8±1.9
Memantine, mg/kg							
10	89.9	55.6	13.3±1.7	11.2±1.4	1.8	17.8	11.2±1.5
20	89.9	33.3	15.8±1.8	10.3±1.2	1.7	17.5	10.4±1.4
IEM-1966, mg/kg							
0.1	75	37.5	22.7±2.6*	12.3±1.5	1.05*	8.75*	7.5*
0.3	50+	12.5+	15.3±1.7	11.0±1.3	0.62+	6.4+	5.4+
1.0	87.5	50	16±1.9	11.7±1.4	2.44	20.8	8.4

Note. *p<0.05, +p<0.02 compared to the control.

TABLE 2. Effect of Course Treatment with IEM-1966 (Postinoculation Days 8-18, Therapeutic Scheme) on Latency, Duration, and Severity of EAE in Female Wistar Rats

Group	Percentage of rats with EAE		EAE latency, days		Mean CI at the peak of EAE, score	Mean cumulative CI, score	EAE duration, days
	total	severe course	mild course	severe course			
Control	100	62.5	12.3±1.5	8.2±0.9	2.56	30.25	15.0±1.9
IEM-1966, mg/kg	62.5	32.5	21.5±2.4*	13.7±1.5	1.19*	13.4*	7.0*

Note. *$p<0.05$ compared to the control.

In 62.5-63.0% control rats, EAE run severe course with paralysis of the extremities and lower part of the body. In these rats, the first symptoms of EAE developed after 8.2-8.9 days (EAE latency), while culmination of the disease developed on days 10-12 and lasted for 6-8 days (Tables 1 and 2).

The control rats with mild or moderate course of EAE demonstrated the first symptoms of the disease in 12.0-12.3 days (latency), while culmination of EAE started on days 12-15 and lasted for 2-5 days. In all control rats, mean CI and mean cumulative CI at the peak of EAE scored 2.2-2.6 and 25.6-30.3, respectively. In these rats, the mean duration of EAE was 15.0-15.8 days (Tables 1 and 2).

The blockers of NMDA-receptors MK-801 and memantine did not increase EAE latency and only slightly alleviated neurological symptoms in rats with EAE even when they were injected at the maximum tolerated doses [5,7,9,11]. In our experiments, memantine injected on postinoculation days 2-16 in doses of 10 and 20 mg/kg prevented the development of EAE in only 10% rats (Table 1). Memantine in both specified doses only slightly (by 1.2-1.3 times) decreased CI at the peak of the disease (Table 1) in comparison with the control rats, and produced no significant effect on cumulative CI. In memantine-treated rats, the duration of EAE decreased by 4-5 days (1.4-1.5 times) in comparison with the control (Table 1), but the latency did not significantly increase.

Thus, our study corroborated the view that individual block of cerebral NMDA-receptors is inefficient for the prevention of the development and moderation of the severity of EAE [6,9,11], because in most rats it did not significantly increase EAE latency and only insignificantly moderated the severity and duration of the disease converting the severe form of EAE into the moderate one.

This study showed that AMPA-blockers exert a more potent neuroprotective effect in rats and mice with EAE probably due to their ability to prevent death of oligodendrocytes and damage to axons and enhance myelin synthesis [11,13]. However, AMPA-blockers cannot decrease BBB permeability and inhibit inflammation in CNS [11,13,15]. Probably, this is why course treatment with selective AMPA-blockers during the induction period of EAE cannot prevent the development of this disease during EAE induction period or significantly increase its latency in rats [11,13].

In hippocampal sections, IEM-1966 blocks both NMDA- and GluR1 AMPA-receptors [3]. In this study, course treatment with IEM-1966 (0.1 mg/kg) completely prevented the development of EAE in 25% rats, while in 37.5% rats the disease developed only after cessation of treatment (latency 22.7 days, $p<0.05$, Table 1) and run mild or moderate course. IEM-1966 in a dose of 0.1 mg/kg significantly decreased the mean cumulative CI (by 2.9 times), CI at the peak of EAE (by 2.1 times), and EAE duration by 2.1 times (Table 1).

After increasing the dose of IEM-1966 to 0.3 mg/kg, EAE developed only in 50% cases ($p<0.02$), while in 37% rats from this group the course of the disease was short and mild (Table 1). IEM-1966 in the specified dose most markedly decreased the severity and duration of EAE (by 3.5-4 times, Table 1). Thus, IEM-1966 injected in doses of 0.1-0.3 mg/kg during postinoculation days 2-16 is not inferior to selective AMPA-blockers by its therapeutic activity, but in contrast to them it can prevent the development of EAE in most rats.

Since the first symptoms of the disease in the rats with severe course of EAE developed as early as on postinoculation days 7-8, the therapeutic course with IEM-1966 encompassed the entire clinical phase up to the end of the disease peak (days 8 through 18). Despite the fact that the therapeutic course of IEM-1966 in a dose of 0.3 mg/kg did not significantly prevent EAE, the percent of rats with EAE trended to decrease, and in 37.5% rats the disease did not develop (EAE developed in 100% control rats). It is noteworthy that the severity of EAE in rats treated with IEM-1966 decreased: the mean cumulative CI decreased by 2.25 times

($p<0.05$, Table 2), CI during culmination of EAE decreased by 2.15 times ($p<0.05$, Table 2), and EAE duration shortened by 2.1 times in comparison with the control. In addition, in rats with mild and moderate course of the disease, the latency increased by 9.2 days ($p<0.05$). However, in 32% rats (severe course of EAE) IEM-1966 (0.3 mg/kg) did not significantly increase the latency, so it could not protect the animals during the entire administration period (Table 2).

Thus, administration of IEM-1966 in a dose of 0.3 mg/kg during postinoculation days 8-18 only partially prevented the development of EAE in 67.5% rats. In other rats, treatment with IEM-1966 by this scheme was not sufficiently effective, because it could not prevent the development of severe EAE. Therefore, administration of IEM-1966 only during the clinical phase is 2-fold less efficient than treatment according to the preventive scheme. In rats with mild and moderate course of EAE, it is sufficient to administer IEM-1966 only during the clinical phase of the disease, although the maximum efficiency is attained when the drug is used during the induction phase of EAE before manifestations of clinical symptoms.

We believe that higher efficiency of IEM-1966 in rats with EAE in comparison with selective NMDA- and AMPA-blockers results not only from its stronger neuroprotective effect due to combined block of cerebral GluR1 AMPA- and NMDA-receptors, but also from its anti-inflammatory effect. This effect originates from a decrease of BBB permeability for encephalitis-producing T-lymphocytes and toxic substances as a result of NMDA-receptor blockade on BBB-forming cells.

Probably, the therapeutic preparations possessing combined NMDA- and GluR1 AMPA-blocking potency will form the basis for novel generation of highly efficient neuroprotective drugs designed for complex treatment of multiple sclerosis.

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