Selective Accumulation of Monoclonal Antibodies against Neurospecific Enolase in Brain Tissue of Rats with Middle Cerebral Artery Occlusion

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Preparations of I^{125}-labeled monoclonal antibodies against neurospecific enolase and mouse plasma IgG1 were injected intravenously to rats immediately after unilateral occlusion of the middle cerebral artery. Radioactivity of I^{125}-labeled monoclonal antibodies against neurospecific enolase in the brain tissue progressively increased, reached a maximum by the 48th hour, and remained practically unchanged after 72 h. At the same time radioactivity of labeled IgG1 in the brain tissue and radioactivity of both preparations in the blood, liver, spleen, kidneys, heart, and lungs decreased over 72 h. Selective accumulation of I^{125}-labeled monoclonal antibodies against neurospecific enolase was less significant in the brain tissue of the contralateral hemisphere and cerebellum not exposed to ischemia.

Key Words: *I^{125}-labeled monoclonal antibodies against neurospecific enolase and mouse IgG1; rats; organ distribution; unilateral occlusion of middle cerebral artery*

Directed transport of bioactive substances and drugs to target nerve cells holds much promise [6,9]. The use of vectorial transport systems is limited by extremely low permeability of the intact blood-brain barrier (BBB) for high-molecular weight compounds [2,4,9]. Cytokines [3], neurotrophic factors [8,10], and antibodies [2,4,7] can penetrate from the blood into the brain tissue under pathological conditions and/or traumas of the central nervous system. It should be emphasized that no more than 0.5% exogenous compounds undergo passage into the brain parenchyma.

Methodological aspects of the experiments, interpretation, and analysis of the results are widely debated topics [2,4,7-9].

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Here we studied the passage, accumulation, and elimination of intravenously injected I^{125}-labeled monoclonal antibodies against neurospecific enolase (^{125}I-Mab NSE) and nonspecific native mouse IgG1 (^{125}I-IgG1) in the brain tissue of rats with unilateral focal cerebral ischemia.

MATERIALS AND METHODS

Monoclonal anti-NSE antibodies were synthesized at the Laboratory of Immunochemistry (V. P. Serbskii State Research Center for Social and Forensic Psychiatry) [1]. Native IgG1 were isolated from mouse plasma by affinity chromatography. Preparations were iodated by the T-chloramine method [2]. Free iodine was removed from ^{125}I-Mab NSE and ^{125}I-IgG1 on Sephadex G-50 microcolumns. The total protein content in preparations was measured using bicinchoninic acid. The amount of Mab NSE was estimated by enzyme immunoassay [1].

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Experiments were performed on 66 adult male Wistar rats weighing 240-270 g. The control group included 20 intact animals. Unilateral middle cerebral artery occlusion (MCAO) was modeled in 46 rats. The animals were kept in the Experimental and Biological Clinic (V. P. Serbskii State Research Center for Social and Forensic Psychiatry) under standard conditions. Surgical manipulations were performed on rats anesthetized with ketamine (100 mg/kg intraperitoneally), Seduxen (50 mg/kg intraperitoneally), and atropine (0.1 ml 1% solution subcutaneously).

Focal cerebral ischemia was modeled by electrocoagulation of the left middle cerebral artery (method of A. Tamura with modifications of J. B. Bederson) [5]. The preparations of ^{125}I-Mab NSE (21 rats) and ^{125}I-IgG1 (25 rats) in a dose of 25 μg (total protein content; 7.5 and 5.3 μCi, respectively) were dissolved in 0.4 ml physiological saline and injected into the femoral vein 10-15 min after occlusion. Intact rats also received ^{125}I-Mab NSE (n=8) and ^{125}I-IgG1 (n=12). To study kinetic characteristics of preparations, blood samples (0.2 ml) were taken from the femoral vein starting from the 1st minute after treatment. The rats were consecutively perfused with 200 ml phosphate buffered saline and 400 ml 4% neutral paraformaldehyde in phosphate buffered saline through the ascending aorta after 3, 48, and 72 h. The liver, spleen, kidneys, heart, and lungs were weighted. Samples (200-300 mg) were taken to measure radioactivity. The brain was weighted, washed 2 times with phosphate buffered saline, dried with filter paper, and placed on a horizontal surface. The cerebellum was excised. The left and right hemispheres were separated along the longitudinal fissure and placed in tubes to study radioactivity.

Radioactivity was measured on a LKB-Wallac 1260 device (MultiGamma) and expressed in cpm. The results were calculated per 1 g weight of organs and/or 1 ml blood taking into account the baseline level of recording. The relative content of a radioactive label was estimated in samples of the blood and organs and expressed in percents of introduced ^{125}I (V_Δ%). Intergroup differences were evaluated by Student's t test. Kinetic characteristics of the radioactive label were determined using Prophet software.

RESULTS

After iodation monoclonal anti-NSE antibodies lost no more than 30% immunochemical activity. Specific radioactivity for Mab-NSE and IgG1 was 0.3 and 0.21 μCi/μg, respectively.

In the blood from rats with MCAO, V_Δ% of ^{125}I-Mab-NSE was much higher than V_Δ% of ^{125}I-IgG1. Similar results were obtained in intact animals (Fig. 1). In rats with MCAO half-estimation times for ^{125}I-IgG1 and ^{125}I-Mab-NSE at the stage of distribution were 17.5 and 89.3 min, respectively and at the stage of elimination 20.5 and 71.3 h, respectively. V_Δ% of ^{125}I-Mab-NSE in the liver, spleen, kidneys, heart, lungs, and brain surpassed V_Δ% of ^{125}I-IgG1 (Table 1). However, no differences were revealed between radioactivity and changes in V_Δ% of ^{125}I-Mab-NSE and ^{125}I-IgG1 in the brain tissue and other organs over 3 days of observations.

In the brain tissue of intact rats V_Δ% for intravenously injected ^{125}I-IgG1 and ^{125}I-Mab-NSE was lower than in other organs (0.005 and 0.007%, respectively, Table 1). Our results are consistent with published data on low permeability of BBB for Ig [2,4,7,9]. We revealed a regular distribution of radioactivity in the left and right hemispheres and cerebellum of intact animals (Table 1).

V_Δ% of ^{125}I-IgG1 and ^{125}I-Mab-NSE in the brain tissue of rats with MCAO increased by more than 10 times 3 h after intravenous injection (0.076 and 0.075%, respectively). Radioactivity was mainly detected in the left ischemic hemisphere (80-85%, Table 1). Radioactivity of ^{125}I-IgG1 and ^{125}I-Mab-NSE in the brain tissue and other organs underwent different changes 48 and 72 h after treatment (Fig. 2). V_Δ% of ^{125}I-IgG1 and ^{125}I-Mab-NSE in the liver, spleen, lungs, heart, and kidneys decreased by more than 2 times compared to 3 h postinjection. In the brain tissue we revealed only a decrease in radioactivity of ^{125}I-IgG1 (Fig. 2). Radioactivity of ^{125}I-Mab-NSE increased by 1.5 times 48 h after injection and remained practically unchanged 72 h after treatment. V_Δ% of ^{125}I-Mab-NSE in the left ischemic hemisphere reached maximum 48 and

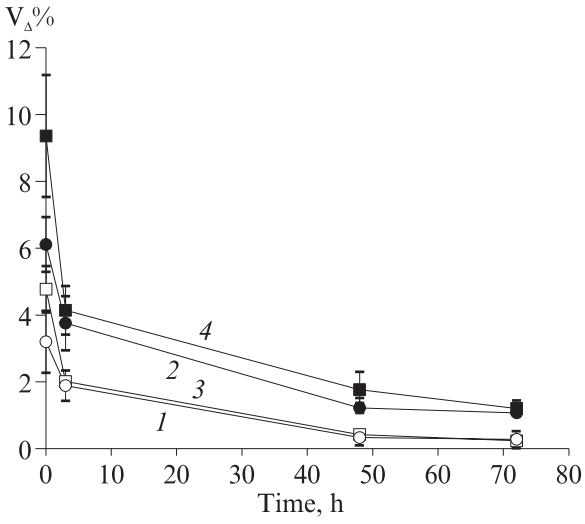

Fig. 1. Radioactivity of the blood in intact rats and animals with unilateral middle cerebral artery occlusion (MCAO) after intravenous injection of ^{125}I-IgG1 and ^{125}I-Mab NSE: intact animals receiving ^{125}I-IgG1 (*1*) and ^{125}I-Mab NSE (*2*); rats with MCAO receiving ^{125}I-IgG1 (*3*) and ^{125}I-Mab NSE (*4*). Here and in Fig. 3: V_Δ%, percent of the introduced label.

TABLE 1. Radioactivity of Organs in Intact Rats and Animals with Left-Sided MCAO after Intravenous Injection of ^{125}I-Mab NSE and ^{125}I-IgG1 (% of the Infected Dose, $\bar{X}_M \pm m$)

Preparations	Brain					Organs, per 1 g tissue				
	1 g tissue	whole brain	hemisphere		cerebellum	liver	spleen	kidneys	lungs	heart
			left	right						
Intact										
3 h ^{125}I-IgG1	0.005±0.001	0.010±0.003	0.003±0.000	0.003±0.000	0.004±0.001	0.198±0.031	0.141±0.025	0.395±0.048	0.070±0.011	0.060±0.015
48 h ^{125}I-IgG1	0.003±0.000	0.005±0.000	0.002±0.000	0.002±0.000	0.002±0.000	0.056±0.007	0.038±0.003	0.090±0.001	0.023±0.002	0.032±0.007
^{125}I-Mab NSE	0.007±0.000**	0.013±0.001**	0.004±0.000**	0.005±0.000**	0.004±0.001*	0.119±0.035	0.102±0.030*	0.141±0.029*	0.138±0.048	0.087±0.049*
72 h ^{125}I-IgG1	0.003±0.000	0.005±0.001	0.002±0.000	0.02±0.005	0.002±0.000	0.039±0.004	0.025±0.002	0.062±0.003	0.020±0.003	0.027±0.004
^{125}I-Mab NSE	0.004±0.000*	0.008±0.001*	0.003±0.000*	0.003±0.000	0.003±0.000	0.080±0.017	0.057±0.005*	0.095±0.011	0.040±0.021	0.054±0.005**
MCAO										
3 h ^{125}I-IgG1	0.040±0.009	0.075±0.015	0.065±0.016	0.006±0.000	0.027±0.021	0.006±0.001	0.185±0.027	0.527±0.060	0.091±0.025	0.091±0.003
^{125}I-Mab NSE	0.044±0.008	0.075±0.010	0.063±0.001	0.006±0.000	0.006±0.001	0.359±0.034	0.196±0.014	0.416±0.019	0.558±0.270	0.232±0.028*
48 h ^{125}I-IgG1	0.023±0.003	0.043±0.006	0.037±0.005	0.004±0.001	0.003±0.000	0.096±0.009	0.055±0.010	0.101±0.012	0.033±0.018	0.044±0.002
^{125}I-Mab NSE	0.061±0.008**	0.115±0.013**	0.098±0.012**	0.008±0.002*	0.006±0.001**	0.169±0.028*	0.099±0.009**	0.122±0.013	0.088±0.012	0.110±0.009**
72 h ^{125}I-IgG1	0.017±0.005	0.034±0.010	0.027±0.009	0.003±0.001	0.003±0.001	0.063±0.005	0.027±0.002	0.079±0.005	0.031±0.005	0.025±0.003
^{125}I-Mab NSE	0.054±0.011*	0.105±0.022*	0.088±0.020*	0.009±0.003	0.007±0.001*	0.120±0.005**	0.064±0.002**	0.127±0.012*	0.054±0.011	0.092±0.012**

Note. *p<0.05 and **p<0.01 compared to ^{125}I-IgG1.

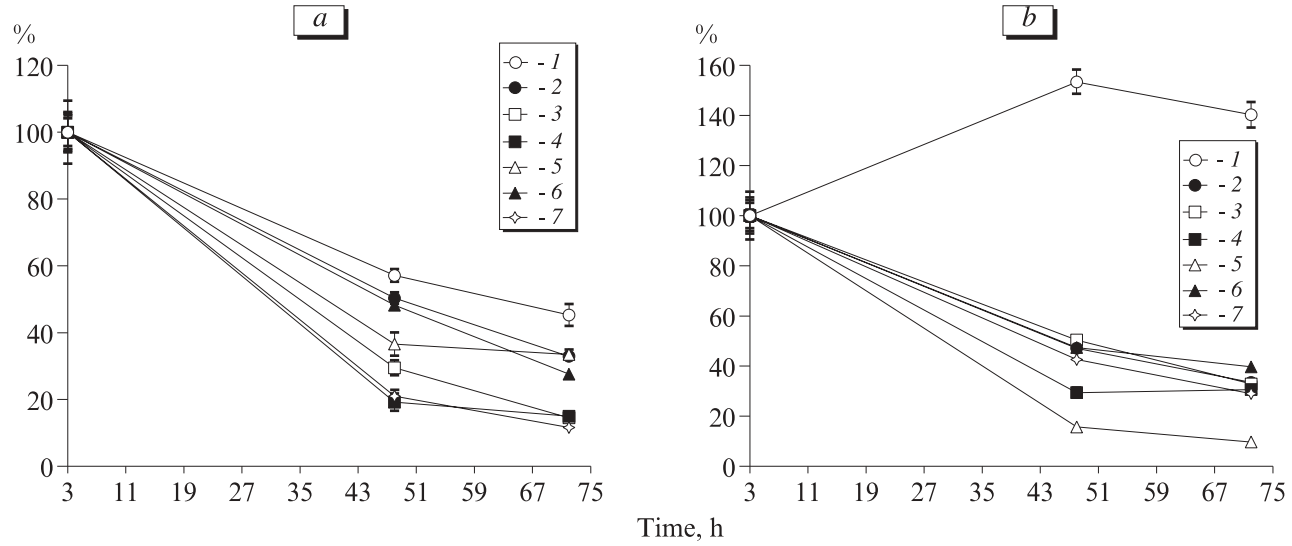

Fig. 2. Relative radioactivity of the brain (*1*), liver (*2*), spleen (*3*), kidneys (*4*), lungs (*5*), heart (*6*), and blood (*7*) in rats with MCAO receiving ^{125}I-IgG1 (*a*) and ^{125}I-Mab NSE (*b*).

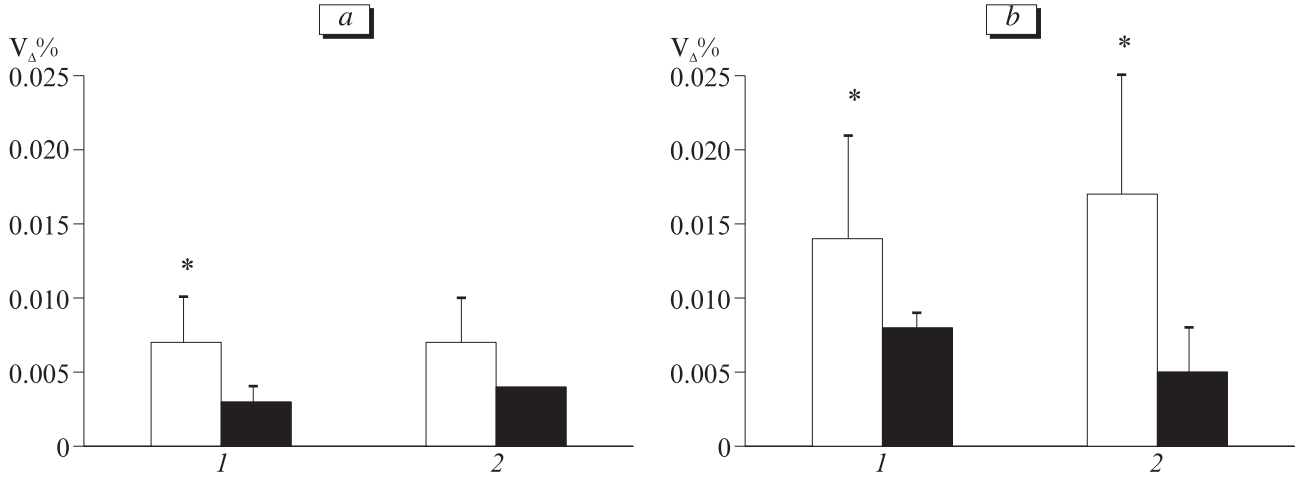

Fig. 3. Radioactivity of nonischemic brain regions in rats with MCAO (light bars) and intact animals (dark bars) after intravenous injection of ^{125}I-IgG1 (*a*) and ^{125}I-Mab NSE (*b*): 48 (*1*) and 72 h postinjection (*2*). **p*<0.05 compared to intact animals.

72 h after treatment (0.16 and 0.15%, respectively). Similar changes were revealed in brain structures not exposed to the direct effect of ischemia (right hemisphere and cerebellum, Fig. 3).

These data show that intravenously injected ^{125}I-IgG1 and ^{125}I-Mab-NSE similarly permeate into rat brain (Fig. 2, 3 h postinjection). In the follow-up period ^{125}I-IgG1 undergo elimination, while ^{125}I-Mab-NSE are accumulated in the brain tissue. We revealed selective accumulation of Mab NSE in the brain tissue (compared to other organs). Mab NSE concentration decreased in the blood, but increased in the brain tissue. Our results illustrate specific binding of Mab NSE to the brain tissue (as differentiated from nonspecific Ig). The observed variations were not related to differences in the kinetics of preparations in the blood (*e.g.*, rapid distribution and elimination of ^{125}I-Mab-NSE).

Our study demonstrated penetration, selective accumulation, and delayed elimination of Mab NSE in the brain parenchyma of rats with experimental ischemic insult. These processes involve not only the ischemic hemisphere, but also brain areas not exposed to ischemia. The data on the permeability of BBB for Ig extend our knowledge about the role of specific autoantibodies in the pathogenesis of nervous system dysfunction accompanied by changes in BBB permeability. Monoclonal antibodies against neurospecific proteins can be used as vectors for directed transport of biologically active substances into the brain.

REFERENCES

1. V. P. Chekhonin, O. I. Gurina, T. S. Portnaya, *et al.*, *Vopr. Med. Khimii*, **48**, No. 5, 477-484 (2002).

2. V. P. Chekhonin, T. B. Dmitrieva, and Yu. A. Zhirkov, *Immunochemical Study of Neurospecific Antigens* [in Russian], Moscow (2000).
3. W. A. Banks, S. A. Farr, and J. E. Morley, *Neuroimmunomodulation*, **10**, No. 6, 319-327 (2002-2003).
4. W. A. Banks, B. Terrell, S. A. Farr, *et al*., *Peptides*, **23**, No. 12, 2223-2226 (2002).
5. J. B. Bederson, L. H. Pitts, M. Tsuji, *et al*., *Stroke*, **17**, No. 3, 472-476 (1986).
6. V. P. Chekhonin, I. A. Ryabukhin, Y. A. Zhirkov, *et al*., *Neuroreport*, **7**, No. 1, 129-132 (1995).
7. S. A. Farr, W. A. Banks, K. Uezu, *et al*., *Life Sci*., **73**, No. 5, 555-562 (2003).
8. W. Pan, W. A. Banks, M. B. Fasold, *et al*., *Neuropharmacology*, **37**, No. 12, 1553-1561 (1998).
9. W. M. Pardridge, *Mol. Interv*., **3**, No. 2, 90-105 (2003).
10. D. Wu and W. M. Pardridge, *Proc. Natl. Acad. Sci. USA*, **96**, No. 1, 254-259 (1999).