Intracarotid L-arginine Reverses Motor Evoked Potential Changes in Experimental Cerebral Vasospasm

Ünal ÖZÜM[1]
Adem ASLAN[2]
Ayşenur TAŞ[3]
H. Zafer KARS[4]

[1,4] Cumhuriyet University School of Medicine, Department of Neurosurgery, Sivas, Turkey
[2] Afyonkarahisar University School of Medicine, Department of Neurosurgery, Afyon, Turkey
[3] Cumhuriyet University School of Medicine, Department of Neurology, Sivas, Turkey

ABSTRACT

OBJECTIVE: To investigate motor evoked potentials during short term L-arginine infusion in cerebral vasospasm after experimental subarachnoid hemorrhage.

METHODS: Three groups were designated for this study: control group, subarachnoid hemorrhage-saline infusion group, and subarachnoid hemorrhage-L-arginine infusion group. A subarachnoid hemorrhage was created by intracisternal injection of autologous blood in New Zealand rabbits. At the fourth day of subarachnoid hemorrhage, latency and amplitude of motor evoked potentials were recorded during intracarotid saline and L-arginine infusion, and compared with motor evoked potential parameters of the control group.

RESULTS: Motor evoked potential latencies were increased, and amplitudes were decreased in all animals before saline and L-arginine infusion on the fourth day of subarachnoid hemorrhage. A decrease in latencies as well as an increase in motor evoked potential amplitudes was observed with short-term intracarotid L-arginine infusion.

CONCLUSION: Intracarotid short term L-arginine infusion significantly improves motor evoked potential parameters after experimental subarachnoid hemorrhage.

KEY WORDS: Cerebral vasospasm, Subarachnoid hemorrhage, L-arginine, Nitric oxide, Evoked potential, Transcranial magnetic stimulation.

INTRODUCTION

Neuronal functions are impaired by insufficient blood supply due to cerebral arterial narrowing or occlusion. Cerebral vasospasm associated with aneurysmal subarachnoid hemorrhage (SAH) is angiographically characterized by luminal narrowing of the proximal and/or distal cerebral arteries. This affects the patient's prognosis by causing decreased global and/or regional cerebral blood flow (CBF), and delayed ischemic neurological deficit (DIND) (5, 17).

Monitoring of regional neuronal and axonal function provides useful data about regional blood supply. Motor evoked potential (MEP) after transcranial magnetic stimulation is an electrophysiological parameter that is used to study the corticospinal motor pathway in clinical and experimental practice. Although MEP has been investigated in different disorders, there is only one report about the result of MEP study during cerebral vasospasm after SAH (10).

Administration of L-arginine, a nitric oxide synthase (NOS) substrate, has been used to increase nitric oxide (NO) production. The

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Correspondence Address
Ünal ÖZÜM
Cumhuriyet University School of Medicine,
Department of Neurosurgery,
Sivas, Turkey
E-Mail: sofamor@gmail.com
Phone: +90 346 2191333

administration of NO, NO donor or NO precursor has caused main cerebral arterial vasodilatation in preclinical studies. However, their effect on neural functional recovery has not been demonstrated (1, 11, 13, 21, 23).

We hypothesized that impaired neuronal/axonal function due to arterial narrowing after SAH can be detected with MEP study. The effect of short-term intracarotid infusion of L-arginine on MEP was investigated in cerebral vasospasm after experimental subarachnoid hemorrhage in the rabbit.

MATERIALS and METHODS

Twenty-four New Zealand albino rabbits of both sexes, weighing between 2350-3050 grams were used in this study. The animals were kept at the Animal Care Facility of Cumhuriyet University Medical Faculty, and the Experimental Research Center through the experiment. Environmental temperature and humidity levels were the same for all animals under normal daylight conditions. The rabbits had free access to food and water through the experiment. The experimental protocol was approved by the Regional Animal Care and Use Committee (01.04.2006-21).

Animal groups: Two groups were designated for this study.

Control group (n=8) and SAH-intracarotid infusion (Car) group (n=16).

Animals in the Car group were randomly assigned to SAH-intracarotid saline infusion (CarS) group (n=8) and SAH-intracarotid L-arginine infusion (CarLA) group (n=8).

Anaesthesia: Intramuscular injections of 50 mg/kg ketamine-HCl plus 5 mg /kg xylazine were given for all surgical procedures. Intramuscular injection of 0.3 mg/kg fentanyl citrate was performed during MEP recording. It was shown that fentanyl citrate does not alter MEP data (8, 9).

Experimental SAH: The modified model of Chan et al. was used to create experimental vasospasm (3). Fresh autologous, non-heparinized blood was drawn from each animal's cannulated femoral artery, and slowly given percutaneously into the cisterna magna with a 24 gauge steel needle at a dose of 0.7 ml/kg after removal of an equal amount of cerebrospinal fluid. The animals were then tilted with tail up for 15 minutes in order to diffuse the blood into cisternal space.

Surgical procedures: In Car groups, an intracarotid catheter was placed on the fourth day of SAH. Under guidance of a surgical microscope, a right paramedian incision extending approximately 3 cm from angle of the mandible inferiorly was made. The right carotid bifurcation was exposed lateral to trachea and esophagus. Both the internal and external carotid arteries were identified. The internal carotid artery was temporarily ligated just above the bifurcation. The internal carotid artery was perforated with 22 gauge steel needle approximately 1 cm superior from the bifurcation. A 2 F silicone catheter (Bard Inc., Murray Hill, NJ, US) was advanced for 2 cm into the internal carotid artery. The catheter was sealed to the artery with tissue glue (Beriplast P, Aventis-Behring, King of Prussia, PA, US) and the incision was closed with layered sutures. Heparinized saline was infused into the catheter in order to prevent coagulation. The tip of the catheter was checked under fluoroscopy. The outer end of the catheter was sealed off and covered with sterile gauze until infusion time.

In Car groups, an intraparenchymal transducer was placed on the fourth day of SAH to monitor intracranial pressure and brain temperature (ICP-BT). A burr hole was opened in the right frontal bone 2 mm anterior to the coronary suture and 3 mm lateral to the midline. After the dura was exposed and perforated, a transducer (Camino 110-4BT, Integra NeuroCare, San Diego, CA, US) was advanced for 5 mm and fixed to the calvarium. A monitoring device (Model MPM-1, Camino with MPM-1 Serial Data Capture Software Version 2.0, Integra NeuroCare, San Diego, CA, US) was used for data recording.

The right femoral artery was percutaneously cannulated in the CarLA group.

MEP recording: The proximal part of each rabbit's right groin was shaved for the MEP recordings. Surface type Ag-AgCl disc electrodes were used. The active electrode was fixed on the skin over the right quadriceps femoris muscle, the reference electrode over the tendon of the same muscle, and the ground electrode on the right ankle. A generator with a maximum output of 2.0 T (Magstim, Long Island City, NY, US) was used in this study. The stimulation coil diameter was 90 mm. All recordings were performed by the same physician. The anatomical reference for the motor cortex was accepted as a point on the midline, 1.5 cm anterior of

the external occipital protuberance. The coil was set in a tangential position to achieve maximum magnetic field over the vertex. Stimulation intensity was adjusted to 80% of maximum output (1.6 T). The final position of the coil was fixed in each animal wherever the evoked response with the greatest amplitude was obtained. In this position, three reproducible MEPs for each animal were recorded with the Neuropack 8 device (Nihon Kohden, Tokyo, Japan). The frequency range was 2 Hz- 3 kHz, sweeping speed 2 ms/division, and sensitivity 500 μV/division. The latency and amplitude of MEPs were analyzed. Cerebral conduction time in the central motor pathway was not used in our study.

Experimental design:

In the control group, MEP latency and amplitude were recorded without performing any surgical procedure and/or infusion.

In Car groups, MEP latency and amplitude were recorded at the fourth day of SAH, and two hours after catheter and ICP-BT transducer placement, the time of which was accepted as the zero minute of MEP recording. After this assessment, sterile saline was infused into internal carotid artery of animals in the CarS group. 300 μmol L-arginine was infused into the internal carotid artery of animals in the CarLA group for 60 minutes (the infusion rate was 1 ml/hour). To prevent any uncontrolled vasoactive effect, an L-arginine solution in water with a pH value of 11.4 was used (Merck Co. Cat No: 101542). During saline and L-arginine infusions, the MEP latency and amplitude were recorded every 10 minutes and MEP recordings were repeated 30 minutes after L-arginine infusion was completed.

Intracranial pressure, intracranial temperature, body temperature, and mean arterial pressure were monitored at 10 minute intervals; arterial PO2, PCO2, HCO3-, SaO2, and pH were monitored at 20 minute intervals in the CarLA groups.

Statistical analysis: The Kruskal-Wallis test, Mann-Whitney U test, and variance analysis of repeated measurements with the Bonferroni test were used for statistical analysis. Pearson's correlation analysis was used to assess relationship between MEP recording amplitude and MEP latency. Significance was accepted at $P<0.05$. SPSS for Windows (Version 10.0) was used.

RESULTS

Alterations in ICP, ICT, BT, MAP, and arterial PO2, PCO2, HCO3-, SaO2 and pH during intracarotid L-arginine infusions were statistically not significant (Table I), ($P>0.05$).

MEP latency recording

Control Group. The mean MEP latency in the control group was 8.53 ± 0.50 msec.

Intracarotid Infusion Group. Table II shows the mean MEP latency values in the CarS and CarLA groups (Table II). There were significant differences between the mean MEP latency values of the control group and the CarS and CarLA groups at the beginning (0 min), respectively ($P<0.05$). Saline infusion did not create any significant MEP latency change during the infusion (Figure 1A) ($P>0.05$). As soon as L-arginine infusion was started, the mean MEP latency value began to decrease and this trend continued until the 20th minute of infusion. Thereafter, the mean MEP latency value showed a stable trend until the end of infusion. There was no

Table I: ICP, ICT, BT, MAP, arterial PO_2, PCO_2, HCO_3-, SaO_2 and pH values with standard error in the *CarLA group*.

Times (Min)	ICP	ICT	BT	MAP	Arterial				
					PO_2	PCO_2	HCO_3-	SaO_2	pH
0 min	6.0±0.26	36.5±0.05	36.5±0.01	86.5±0.26	92.1±0.05	31.3±0.01	24.2±0.03	96.1±0.22	7.43±0.05
10 min	5.1±0.22	36.5±0.02	36.7±0.14	88.0±0.26					
20 min	4.5±0.18	36.6±0.03	36.5±0.02	89.1±0.22	90.5±0.11	30.6±0.02	23.5±0.04	95.0±0.26	7.40±0.05
30 min	4.8±0.22	36.5±0.03	36.5±0.01	87.0±0.32					
40 min	5.1±0.22	36.7±0.03	36.8±0.03	88.6±0.26	92.4±0.07	28.7±0.05	24.7±0.04	95.1±0.22	7.42±0.04
50 min	6.1±0.22	36.6±0.03	36.6±0.01	85.0±0.26					
60 min	6.2±0.25	36.7±0.04	36.9±0.02	88.1±0.51	88.3±0.10	30.9±0.05	24.1±0.06	94.6±0.32	7.43±0.04

ICP: Intracranial pressure (mmHg), ICT: Intracranial temperature (C°), BT: Body temperature (C°), MAP: Mean arterial pressure (mmHg), PO_2 Pressure of O_2 (mmHg), PCO_2 Pressure of CO_2 (mmHg), HCO_3- Bicarbonate concentration (mmol/lt), SaO_2 Oxygen saturation (%), pH Blood pH.

Table II: Mean MEP latency and amplitude values with standard error in the *CarS* and *CarLA* groups.

	CarS group		CarLa group	
	MEP latency	MEP amplitude	MEP latency	MEP amplitude
Times (min)	msec ± Se	mV ± Se	msec ± Se	mV ± Se
0 min	11,72±0,77	403,75 ±13,88	11,82±0,51	413,25±54,12
10 min	11,65±0,65	403,60±20,85	10,43±0,42	599,00±73,62
20 min	11,78±0,66	402,80±21,65	9,40±0,37	780,00±92,44
30 min	11,68±0,68	400,50±18,25	8,94±0,46	845,75±57,77
40 min	11,65±0,65	401,65±21,25	8,59±0,59	914,62±201,98
50 min	11,60±0,77	405,65±19,50	8,37±0,67	912,62±99,13
60 min	11,59±0,50	407,00±24,64	8,25±0,51	919,12±64,69
90 min			11,16±0,38	545,62±56,61

MEP motor evoked potential, msec milisecond, Se standard error, mV milivolt.

significant difference between the mean MEP latency value in the control group and the mean MEP latency value at the 20th, 30th, 40th, 50th, 60th minutes in CarLA group, respectively (P>0.05).

The mean MEP latency thirty minutes after the infusion was completed (90th minute of experiment) was higher than the mean value at the 60th minute but lower than the mean value at the 0 minute in the CarLA group (Figure 1A). The mean MEP latency value recorded 30 minutes after the infusion was completed was significantly different than the mean MEP latency values of all minutes in the CarLA group (P<0.05).

MEP amplitude recording

Control Group. The mean of MEP amplitude in the control group was 826.12 ± 47.39 mV.

Intracarotid Infusion Group. Table II shows the mean MEP amplitude values in the CarS and CarLA groups (Table II). There were significant differences between the mean MEP amplitude values of the control group and the CarS and CarLA groups at the beginning (0 min), respectively (P<0.05). Saline infusion did not create any significant MEP amplitude change during the infusion (Figure 1B) (P>0.05). As soon as L-arginine infusion was started, the mean MEP amplitude value began to increase and this trend continued until the 20th minute of infusion. Thereafter, the mean MEP amplitude value showed a stable trend until the end of infusion. There was no significant difference between the mean MEP amplitude value in the control group and the mean MEP amplitude value at the 20th, 30th, 40th, 50th, 60th minutes in the CarLA group, respectively (P>0.05).

The mean MEP amplitude thirty minutes after the infusion was completed (90th minute of experiment) was lower than the mean value at the 60th minute, but higher than the mean value at the 0 minute in the CarLA group (Figure 1B). The mean MEP amplitude value recorded 30 minutes after the infusion was completed was significantly different from the mean MEP amplitude values of all minutes in the CarLA group (P<0.05).

DISCUSSION

Morton and Merton reported the first experience in recording the evoked response after transcranial electrical stimulation of the motor cortex via the scalp (18). They recorded electromyographic response in the distal muscle after transcranial stimulation. The first use of transcranial magnetic stimulation in an electrophysiological experiment was reported by Barker et al. in 1985 (2). In recent years, developments in specific magnetic coils that can stimulate specific cortical area have made use of magnetic stimulation in investigation of specific corticospinal tracts possible (6). Kawai et al. explored the origins and conducting pathways of MEPs elicited by transcranial magnetic stimulation in cats (14).

MEPs after transcranial magnetic brain stimulation are used for noninvasive evaluation of the corticospinal motor system. MEPs can be used

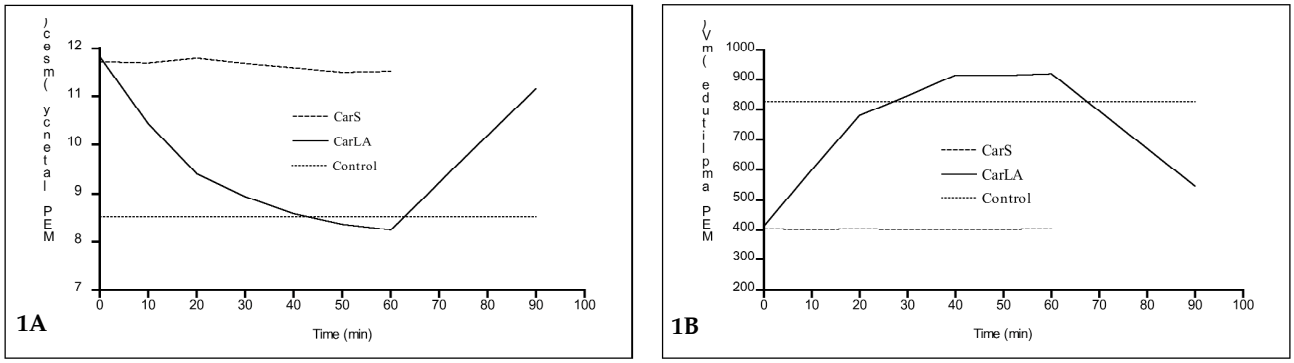

Figure 1. Mean MEP latency (**A**) and mean MEP amplitude (**B**) curves in CarS and CarLA groups. The interrupted line shows the mean MEP latency of the control group. While intracarotid saline infusion did not create any significant mean MEP latency and amplitude change during the infusion (P>0.05), intracarotid L-arginine infusion caused a decrease in mean MEP latency (**A**) and an increase in mean MEP amplitude (**B**) until the 20th minute of infusion (P<0,05). After the 20th minute of infusion, a stable trend was observed during the rest of L-arginine infusion (**A, B**). Although an increase in mean MEP latency (**A**) and decrease in mean MEP amplitude (**B**) was observed 30 minutes after the infusion was completed, the mean MEP latency value was higher than the mean value at the 60th minute, but lower than the mean value at the 0 minute in CarLA group (**A**) and the mean MEP amplitude value was lower than the mean value at the 60th minute, but higher than the mean value at the 0 minute in CarLA group (**B**) (P<0.05).

for the diagnosis and monitoring of clinical and subclinical abnormalities in motor neuron disease, muscle disorders, multiple sclerosis, ischemic brain injury and spinal cord diseases (4, 7, 16, 19, 24, 27). Magnetic stimulation studies can specifically address the issue of cortical reorganization by mapping procedures with focal stimulations using specific coils (12, 25). In addition to clinical use, different MEP modalities have been tried in different experimental studies (10, 13, 15, 22, 26).

There is only one experimental study in which changes in MEP after SAH were recorded. Göksel et al. recorded MEP changes after deferoxamine treatment during cerebral vasospasm after experimental SAH. Cerebral angiography was used to evaluate cerebral vasospasm in this study. Only latency values obtained from MEP study were used and amplitude values were omitted due to extreme variation (10).

L-arginine has been used in several studies. It has been reported that the injection of L-arginine into the cisterna magna caused significant vasodilatation within short time (11, 21). Göksel et al. preferred continuous intracisternal infusion of L-arginine in order to eliminate the short action time disadvantage of L-arginine. Intracisternal L-arginine infusion can improve cerebral vasospasm (11).

In this study changes in latency and amplitude of MEP after SAH were examined. In a previous study it was shown that continuous intracisternal and intracarotid L- arginine infusion improves mean flow velocity (MFV) value in experimental cerebral vasospasm via transcranial Doppler ultrasound (TCD) (20). In this report it is also stated that intracarotid L-arginine infusion is more potent and safer since large amounts of intracisternal L-arginine can lead to overproduction of NO by inducible NOS, which may trigger the production of free radicals. Therefore only intracarotid L-arginine was used in this study.

At the 4th day of SAH and before L-arginine infusion, significantly increased MEP latencies and decreased MEP amplitudes were observed. These changes in MEP values may be related to cerebral ischemia during vasospasm. Although cerebral blood flow normalized at the 30th minute of intracarotid L-arginine infusions in the previous study, MEP latencies and amplitudes improved at the 20th minute of infusions in this study (20). One of the possible explanations of this phenomenon is the early and/or additional effect of intracarotid L-arginine infusion on cortical microcirculation. The second explanation is early and/or sufficient cortical blood supply. The main cerebral arterial blood flow did not return to its normal value before the 20th minute of intracarotid L-arginine infusion. However, blood flow in the main arteries may be sufficient to maintain neuronal and axonal functions at this time. This is the basis of the difference between clinical and angiographic vasospasm. In this study, the effect

of short term L-arginine infusion on motor evoked potential parameters in experimental cerebral vasospasm in a rabbit subarachnoid haemorrhage model was investigated. Intracarotid infusion of L-arginine significantly improved MEP parameters after SAH. This preliminary study suggests that continuous intracarotid infusion of L-arginine, a substrate of NOS, can be used for treatment of cerebral vasospasm. Future studies, which investigate the effect and safety of various doses and durations of intracarotid L-arginine infusion in cerebral vasospasm after SAH may lead to new treatment models for vasospasm following SAH.

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