AMINOGUANIDINE REDUCES INFARCT VOLUME AND IMPROVES NEUROLOGICAL DYSFUNCTION IN TRANSIENT MODEL OF FOCAL CEREBRAL ISCHEMIA IN RAT

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ABSTRACT

Focal cerebral ischemia (Stroke) is the cessation or severe reduction of blood flow to an area of the brain that through activation of a complex cytotoxic cascade results in neuronal cell death. The present study was designed to examine the effects of post-ischemic treatment with aminoguanidine (AG) on cortical, striatal infarct volume as well as neurological dysfunctions. Rats (n=23) were allocated to sham, saline or AG (300 mg/kg)-treated groups. Ischemia was induced by 90 minutes middle cerebral artery occlusion, followed by 24 hrs reperfusion. Saline or AG was administered intraperitoneal at one hour after induction of ischemia. At the end of 24hrs reperfusion, neurological deficit score was tested and cortical, striatal infarct volumes were determined by Triphenyltetrazolium chloride staining. Administration of AG (300 mg/kg) at one hours after ischemia resulted in a significantly lower cortical (85±25 vs. 210±13 mm^3), striatal (35±5 vs. 58±10 mm^3) infarct volumes, and neurological deficit score (1.88±0.23 vs. 2.67±0.21).

Our findings indicate that aminoguanidine is a potent neuroprotective in rat model of transient focal cerebral ischemia. The future studies are required to clear cerebroprotective mechanism of aminoguanidine and possible use of this agent as a therapeutic target in stroke patients.

Keywords: Aminoguanidine, Focal cerebral ischemia, Rat

INTRODUCTION

Aminoguanidine (AG) is a nontoxic small molecule currently under investigation for the treatment of diabetic nephropathy (1). In experimental studies, aminoguanidine was shown to prevent diabetic nephropathy (2), retinopathy (3) and neuropathy (4), as well as atherogenesis (5) and impairment of learning behavior and hippocampal long-term potentiation (6). Moreover, it prevents the formation of advanced glycation end products (AGEs), and inhibits inducible nitric oxide synthase and diamine oxidase (7).

A number of studies using various designs and protocols have shown that AG is neuroprotective. In one such studies (8) a protocol of 2 hrs ischemia and 94 hrs reperfusion was used, and AG was give for 3 days starting 24 hrs after the onset of ischemia. The study showed that AG did reduce the infarct volume (8). A more recent study (9), which employed permanent ischemia protocol, used daily administrations of AG starting 24 hrs after ischemia and determined infarct volume at 48 or 96 hrs post ischemia. Results showed that AG did cause the reduction of infarct volume at 96, but not 48 hrs post ischemia. A fairly recent study (10) did use the protocol of 2 hrs ischemia and 48 and 72 hrs reperfusion, administered AG at 6, 24 and 48 hrs after the onset of ischemia, and determined the infarct volume at 46 and 70 hrs post ischemia. The study (10) revealed that AG did reduce the infarct volume in upper and lower cortex and caudate putamen significantly at 70, but not 46, hrs post ischemia.

As far as the literature is concerned, previously published reports did study the effects of late treatment with AG on neocortex or total cerebral infarct size have been studied mainly in permanent ischemia. However, effects of the early treatment with AG on cortical and striatal infarct volumes as well as neurological dysfunctions have not yet been investigated. Therefore, the present study was designed to investigate the effects of AG on these parameters in a rat model transient focal cerebral ischemia.

MATERIALS AND METHODS

Animals

Experiments were performed in conformity with the university research council guidelines for conducting animal studies. Male Sprague-Dawley rats (Razi institute, Shiraz, Iran) were housed in standard cages at a temperature of 22-24 ºC, humidity of 40-60% and light period o 07.00-19.00 hr controlled environment. Rats had free access to water and food pellets.

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Instrumentation and surgical procedures

Rats (285-350 g) were anesthetized with intraperitoneal (ip) injection of chloral hydrate (400 mg/kg). Trachea was intubated through the mouth to prevent animal's asphyxia and hypoxia. The right femoral artery cannulated for continuous recording of blood pressure and removal of blood samples for blood gas analysis. Afterwards, as reported previously (11), under an operation microscope, right common carotid artery (CCA) and external carotid artery (ECA) was exposed, and carefully dissected free from vagus nerve and surroundings. The internal carotid artery (ICA) was then dissected to the level petrigopalatine artery (PA). Then PA was ligated, a silk thread placed loosely around the ECA stump, and CCA and ICA were occluded temporarily with a microvascular clip. A small incision was then made on ECA, and the Poly-L-Lysine-coated nylon (3-0) thread was passed through it. While holding the thread around ECA tightly to prevent bleeding, the microvascular clip on ICA was removed, and the nylon thread carefully and slowly pushed forward through ICA. Depending on weight, an advance for 20-22 mm from CCA bifurcation placed the tip of nylon thread at the beginning of anterior cerebral artery, resulting in the occlusion of middle cerebral artery (MCA, Fig 1). Rectal temperature was measured by a thermometer and maintained at 37±0.5ºC throughout the experiment by a heating lamp.

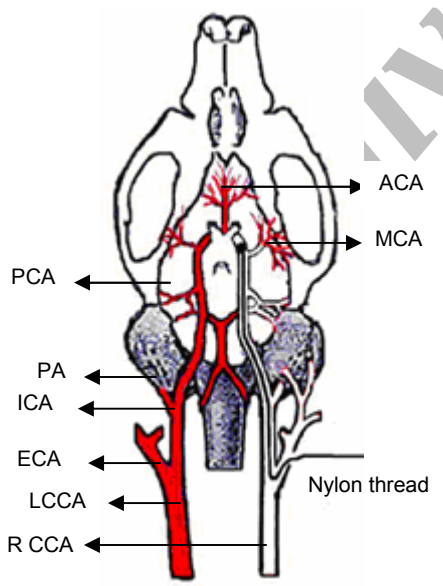

Figure 1. Schematic representation of rat brain arteries and nylon thread used to make middle cerebral artery occlusion and induce of focal cerebral ischemia.
LCCA= Left common carotid artery, RCCA= Right common carotid artery, ECA=External carotid artery, ICA=Internal carotid artery, PA= Pterygopalatine artery, MCA= Middle cerebral artery, BA=Basilar artery

Experimental design

Rats (n =23) were randomly assigned to three groups. The first group was a sham (n=6) receiving no vehicle or drug. The second group (n=9) was MCA occluded rats receiving an ip injection (1ml/kg) of normal saline as the vehicle. The third group (n=8) was MCA occluded rats receiving an ip injection of 300 mg/kg AG (Sigma Chemicals, UK) after one hours of MCA occlusion, 30 minutes before the start of reperfusion phase.

Experimental protocol

All of the surgical procedures and blood sampling, except MCA occlusion, were performed on sham operated group. After recuperation from surgical stress, a measurement of blood pressure was made and a blood sample was withdrawn for the assessment of blood pH and gases. Ten minutes later, in the second and the third groups, ischemia phase (90 minutes) was induced by occluding MCA using intraluminal nylon thread method(11). One hour after initiation of ischemia phase, animals received saline (1 ml/kg, ip) or AG (300 mg/kg, ip). Ninety minutes after induction of ischemia, the reperfusion phase (24 hrs) was started by removal of the nylon thread from MCA. Ten minutes after the start of reperfusion phase, an arterial blood pressure was recorded and a blood sample was withdrawn for the measurement of blood glucose, pH and gases. Animals were kept in a warm place until recovered from anesthesia, then each one was kept in a single cage for 24 hours reperfusion phase, after which neurological deficit test was performed. Animals were then sacrificed, and their brains collected for determination of infarct volumes.

Evaluation of neurological deficits

Evaluation of neurological deficits was performed using five point scoring system as described (12). Accordingly scoring were performed as follows; normal motor function = 0, flexion of contralateral torso or forelimb upon lifting by tail or failure to extend forepaw when suspend vertically = 1, circling to the contralateral side but have normal posture at rest = 2, loss of righting reflex = 3, and no spontaneous motor activity = 4.

Measurement of infarct volume

Rats' brains were cleansed gently and immersed at 4 ^{o}C cold saline for 5 minutes. They were then sectioned coronally into six 2-mm thick slices using a Brain Matrix. Afterwards, slices were immersed in 2% Triphenyltetrazolium chloride solution (Sigma Chemicals, UK), and kept at 37°C in a water bath for 15 minutes. The slices were then transferred to 10% buffered formalin (Merck, Germany). Twenty-four hour later, slices were photographed using a digital camera connected to a computer (Cannon, Japan; Figure 2).

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Infarct areas were measured (in mm^2) using an Image Analyzer Software (NIH Image Analyzer). The infarct volume (mm^3) of each slice was calculated by multiplying infarct area and its thickness (2-mm). The total infarct volume of each brain was then calculated as the sum of the infarct volumes of the six brain slices. Since brain edema might significantly affect the accuracy of infarct estimation (13, 14), the infarct volumes were corrected for brain edema using the following formula (13):

Corrected infarct volume = Left hemisphere volume (non-ischemic hemisphere) – (Right hemisphere volume (ischemic hemisphere) – Measured infarct volume).

Statistical analysis

Data are presented as Mean±SEM. Infarct volumes were compared using Student's t-test. Neurological deficit scores and physiological parameters such as MAP, $PaCO_2$, PaO_2, blood pH and glucose levels were analyzed using Mann-Whitney Rank Sum Test. Statistical significance was set at $P < 0.05$.

RESULTS

There were no significant differences between physiological variables, including MAP, $PaCO_2$, PaO_2, blood pH and glucose levels from rats subjected to sham-operation, MCAO receiving saline or AG (table 1). The sham-operated rats did not have cortical or striatal infarct volumes (Fig 2A), whereas in saline-treated group these volumes were 210±13 mm^3 and 58±10 mm^3, respectively (Fig 2B). Relative to vehicle-treated group, the AG-treated group had a significantly lower cortical (85±25 mm^3) and striatal (35±5 mm^3) infarct volumes (Fig 3A and 3B).

While the neurological deficit scores of sham-operated rats was zero, in AG–treated group this score (Fig 3C) was 1.88±0.23, which was significantly lower than that of saline-treated group (2.67 ± 0.21).

DISCUSSION

The aim of this study was to evaluate the neuro-protective effects of AG on the early stage ischemic damage and neurological deficit in a rat model of transient focal cerebral ischemia. Transient focal cerebral ischemia of the MCA was induced in rat by an intraluminal filament, a

reliable and highly reproducible method which is routinely used in our laboratory (11)

We found that treatment with AG, given one hour after induction of ischemia, resulted in reductions of 60% and 40% in cortical and striatal infarct volumes, respectively. This finding is in agreement with previous observations that AG significantly reduced infarct volume when given at 60 minutes (88%) and 120 minutes (85%) after permanent focal cerebral ischemia in rat (15).

The protective effect of AG in this study cannot be related to changes in arterial pressure, blood gases, rectal temperature, since these parameters which were carefully monitored were not significantly different among the three groups. Moreover, the protection exerted by AG cannot be related to its effects on cerebral blood flow because previous study has demonstrated that AG does not affect resting cerebral blood flow (16). Moreover, it is shown that AG partially inhibits the inducible isoform of Nitric Oxide Synthase (iNOS), and by this maechanism might have neuroprotection action.(17) However, it is unlikely that the reduction in the infarct size by AG was related to iNOS inhibition in this study, because, it has been demonstrated that iNOS expression does not increase during the first 24 hours after the onset of ischemia (18). In this study, aminoguanidine was administered one hour after initiation of ischemia and infarct volume was measured 24 hours after ischemia, before iNOS protein was increased. Thus, it is likely that the observed neuroprotective effects of AG in this study is related to other effects of AG.

Another likely mechanism of neuroprotective actions of AG might be its free radical scavenging or antioxidant capabilities. In vitro studies have demonstrated that AG scavenges free radicals such as H_2O_2 and peroxynitrite in vitro (19), and prevents formation of hydroxyl radical and inhibits peroxidation of lipids and oxidant-induced apoptosis (20). Since, free radicals and apoptosis are believed to play an important role in brain ischemic injuries (21, 22), some of the neuroprotective effects of AG might be justified.

Moreover, part of neuroprotection offered by AG might also be related to its ability to inhibit diamine oxidase (15), an enzyme that participates in the degradation of histamine and polyamines (23). The terminal oxidative deamination of polyamines by diamine oxidase leads to production of toxic aldehydes (23), which could potentially contribute to cerebral ischemic damage. Because increased polyamine synthesis and degradation begins early after an ischemic insult (24), it is likely that inhibition of diamine oxidase contributes to the protective effect of AG.

Another mechanism by which AG caused neuro-protection might be inhibition of polyamine oxidase (PAO). The inhibition of this enzyme caused reduction of a toxic by-product, 3-aminopropanal which results in neuroprotection of ischemic brain damage (25).

It was also shown that administration of AG significantly improved neurological deficits after 90 min ischemia and 24 hours reperfusion in rat. More importantly, in the present study a relationship between the decreased brain damage and improvements of neurological deficits that associated with MCA occlusion was demonstrated. Although, there is no information about the early effect AG treatment on the

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Table 1. Values are mean ± SEM. Mean arterial pressure (MAP, mmHg), pH, $PaCO_2$ (mmHg), PaO_2 (mmHg) and Blood glucose (BG, mg/dl) in sham-group, and rats with middle cerebral artery occlusion receiving 1 ml/kg normal saline or 300 mg/kg aminoguanidine(AG) at 10 minutes before and 10 minutes after middle cerebral artery occlusion.

Variables	**Sham**	10 min before MCAO		10 min after MCAO	
		Saline	**AG**	**Saline**	**AG**
PH	7.35± 0.02	7.34± 0.03	7.28 ± 0.0 2	7.30 ± 0.04	7.26 ± 0.0 3
P_aCO2	35±6	36 ± 7	40 ± 2	36 ± 3	38 ± 5
P_aO2	78±7	82 ± 4	79 ± 3	78 ± 6	80 ± 20
MAP	94±5	91± 7	97 ± 7	90 ± 9	90 ± 4
(BG, mg/dl)	151±12	213 ± 19	192 ± 26	197 ± 17	160 ± 24

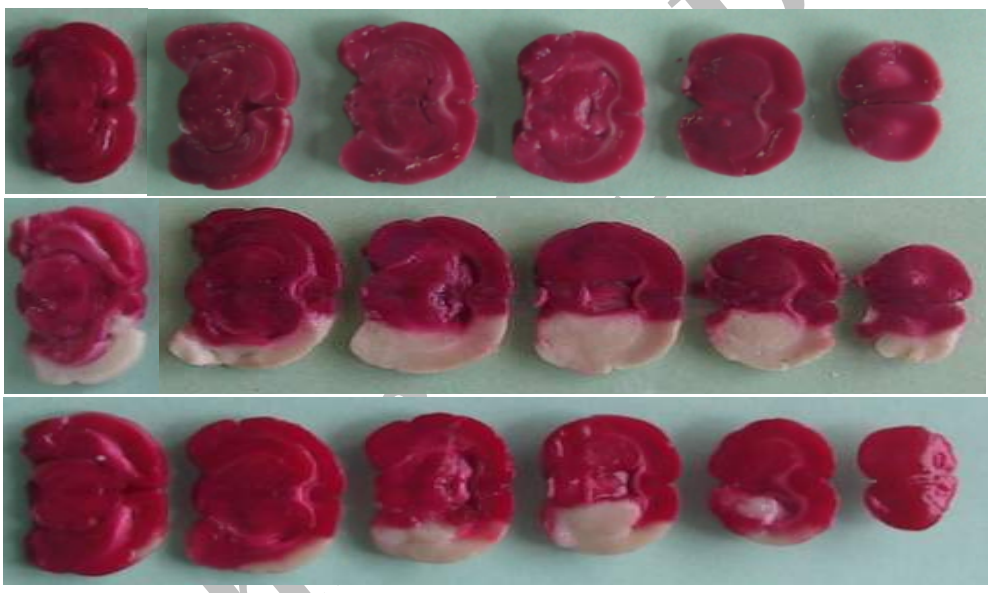

Figure 2. Photographs illustrating the Coronal sections of rat brain with TTC staining, after 90 min MCAO and 24 hours reperfusion, in which red color is normal area and white color is infarct area. Colorless region corresponds to occluded MCA territory. A. Sham group, B. Saline-treated group and C. AG - treated group.

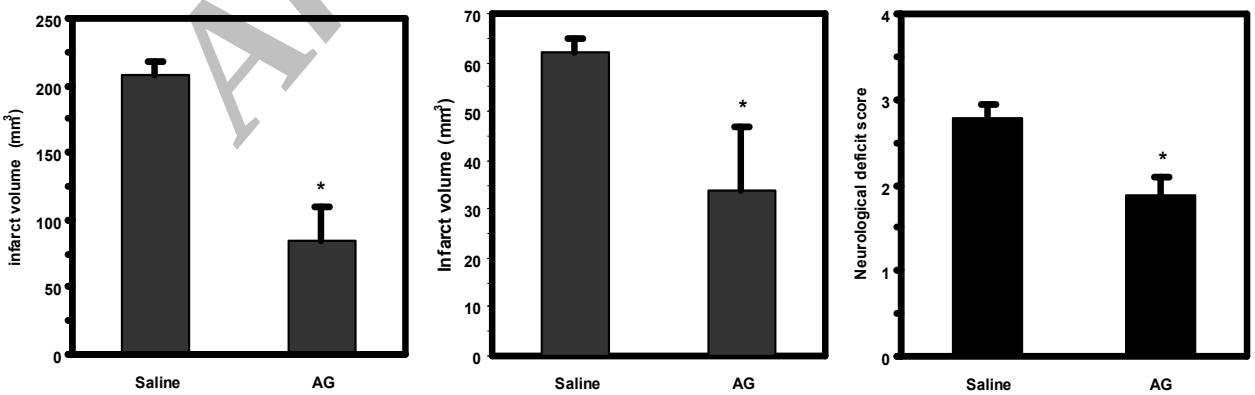

Figure 3. A. Cortical infarct volume, B. Striatal Infarct volume and C. neurological deficit score of rats receiving saline or AG at dose 300 mg/kg at 60 min after ischemia. Values are mean ± SE for each group. * denotes significant difference from saline group ($p<0.05$)

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neurological deficits but delayed treatment with AG, 24 hours after ischemia, led to significant improvement in neurological deficits as well as adecrease in cerebral infarct volume under permanent MCA occlusion (9).

In conclusion, present study shows that aminoguanidine has protective effects in rat model of focal transient cerebral ischemia induced by occlusion of middle cerebral artery. These findings should be taken as an evidence supporting validity of aminoguanidine as a therapeutic potential in the early stages of cerebral ischemia. Therefore, more experimental and clinical studies are required to elucidate the cerebroprotective mechanisms and likely therapeutic indications of aminoguanidine.

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REFERENCES

1. Abdel-Rahman E, Bolto WK. Pimagedin: a novel therapy for diabetic nephropathy. Expert Opin Investig Drugs. 2002; 11:565-74.
2. Taguchi T., Miyoshi H, Sugiura M., Takeuchi M., Yanagisawa K, Watanabe Y, Miwa I, Makita Z. A glycation inhibitor, aminoguanidine and pyridoxal adduct, suppresses the development of diabetic nephropathy. International congress series 2002; 1245: 435-437.
3. Hammes HP, Martin S, Federlin K, Geisen K, Brownlee M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. Proc Natl Acad Sci USA 1991; 88: 11555-8. Erratum in: Proc Natl Acad Sci USA 1992; 89: 9364.
4. Yagihashi S, Kamijo M, Baba M, Yagihashi N, Nagai K. Effect of aminoguanidine on functional and structural abnormalities in peripheral nerve of STZ-induced diabetic rats. Diabetes 1992; 41: 47-52.
5. Panagiotopoulos S, O'Briean RC, Bucala R, Cooper ME, Jerums G. Aminoguanidine has an anti-atherogenic effect in the cholesterol-fed rabbit. Atherosclerosis 1998; 136: 125-131.
6. Mori K, Togashi H, Ueno K-I, Matsumoto M, Yoshioka M. Aminoguanidine prevented the impairment of learning behavior and hippocampal long-term potentiation following transient cerebral ischemia. Behavioral Brain research 2001; 120: 159-168.
7. Nilsson BO. Biological effects of aminoguanidine: an update. Inflammation Research 1999; 48 : 509-15.
8. Zhang F, Robyn M, Casey BS, Ross ME, Iadecola C. Aminoguanidine ameliorates and L-arginine worsens brain damage from intraluminal middle cerebral artery occlusion. Stroke 1996; 27: 317-323.
9. Nagayama M, Zhang F, Iadecola C. Delayed treatment with aminoguanidine decreases focal cerebral ischemic damage and enhances neurologic recovery in rats. J. Cerebral Blood Flow Metab. 1998; 18:1107-13.
10. Cash D, Beech JS, Rayne RC, Bath PM, Meldrum BS, Williams SC. Neuroprotective effect of aminoguanidine on transient focal ischaemia in the rat brain. Brain Res. 2001; 29: 91-103.
11. Vakili A, Nekooeian AA, Dehghani GA. L-NAME and 7-nitroimmidazole reduces brain injuries in transient focal cerebral ischemia in rat. Iran J of Med Sci 2004, 29 : 109-115
12. Vakili A, Kataoka H, Plesnila N. Role of arginine vasopressin V1 and V2 receptors for brain damage after transient focal cerebral ischemia. J. Cerebral Blood Flow Metab. 2005; 25, 1012-1019.
13. Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR. Semiautomated method for measuring brain infarct volume. J. Cerebral Blood Flow Metab. 1990; 10: 290-3.
14. Brint S, Jacewicz M, Kiessling M, Tanabe J, Pulsinelli W. Focal brain ischemia in the rat: methods for reproducible neocortical infarction using tandem occlusion of the distal middle cerebral and ipsilateral common carotid arteries. J. Cerebral Blood Flow Metab. 1988; 8: 474-85.
15. Cockroft KM, Meistrell M, Zimmerman GA, Risucci D, Bloom O, Cerami A, Tracey KJ. cerebroprotective effects of aminoguanidine in a rodent model of stroke. Stroke 1996; 27: 1393-8.
16. Iadecola C, Zhang F, Xu S, Casey R, Ross ME. Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. J. Cerebral Blood Flow Metab. 1995; 15:378-84.
17. Wolff DJ, Lubeskie A. Aminoguanidine is an isoform-selective, mechanism-based in activator of nitric oxide synthase. Arch Biochem Biophys. 1995 ; 316:290-301
18. Iadecola C, Zhang F, Casey R, Nagayama M, Ross ME. Delayed reduction of ischemic brain injury and neurological deficits in mice lacking the inducible nitric oxide synthase gene. J Neurosci. 1997; 17:9157-64.
19. Yildiz G, Demiryurek AT, Sahin-Erdemli I, Kanzik I. Comparison of antioxidant activities of aminoguanidine, methylguanidine and guanidine by luminol-enhanced chemiluminescence. Br J Pharmacol. 1998; 124: 905-10.

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20. Giardino I, Fard AK, Hatchell DL, Brownlee M. Aminoguanidine inhibits reactive oxygen species formation, lipid peroxidation,and oxidant-induced apoptosis.Diabetes 1998; 47: 1114-20.
21. Gilgun-Sherki Y, Rosenbaum Z, Melamed E, Offen D. Antioxidant therapy in acute central nervous system injury: current state. Pharmacol Rev 2002; 54: 271-84.
22. Plesnila N, Zhu C, Culmsee C, Groger M, Moskowitz MA, Blomgren K. Nuclear translocation of apoptosis-inducing factor after focal cerebral ischemia. J Cereb Blood Flow Metab 2004; 24: 458-66.
23. .Sessa A, Perin A. Diamine oxidase in relation to diamine and polyamine metabolism. Agents and Actions 1994; 43: 69-77.
24. Paschen W, Cleef M, Rohn G, Muller M, Pajunen AE. Ischemia-induced disturbances of polyamine synthesis. Prog Brain Res. 1993; 96: 147-60.
25. Ivanova S, Botchkina GI, Al-Abed Y, Meistrell M 3rd, Batliwalla F, Dubinsky JM, Iadecola C, Wang H, Gregersen PK, Eaton JW, Tracey KJ. Cerebral ischemia enhances polyamine oxidation: identification of enzymatically formed 3-aminopropanal as an endogenous mediator of neuronal and glial cell death. J Exp Med. 1998; 188:327-40.

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