EFFECT OF AMINOGUANIDINE ON POST-ISCHEMIC DAMAGE IN RODENT MODEL OF STROKE

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ABSTRACT

A number of studies have shown that aminoguanidine (AG) has neuroprotective effect in chronic phase of cerebral ischemia. However, dose dependent effects of AG on acute phase of ischemic-reperfusion injuries, as well as neurological dysfunctions are not completely clear. Transient focal cerebral ischemia was induced in rats by 60 min middle cerebral artery occlusion (MCAO), followed by 23 h reperfusion. Saline as vehicle or AG at doses 75, 150, or 300 mg/kg ip was administered at the beginning of ischemia. Infarct volume and motor dysfunction were assessed 24 h after MCAO. Treatment with AG at dose 75, 150, or 300 mg/kg ip significantly reduces total infarct volumes by 44%, 56% and 36%, respectively. In addition, AG only at dose 150 significantly improves neurological dysfunction in comparison with saline group. Our findings show that AG decrease ischemic brain damage dose-dependently and improve neurological recovery in acute phase of transient focal cerebral ischemia. More studies are needed to find therapeutic window and mechanisms of neuroprotection of AG in early phase of cerebral ischemia.

Keywords: Aminoguanidine, Acute phase, Transient Focal cerebral ischemic, neurological dysfunction.

INTRODUCTION

Several experimental studies have shown that aminoguanidine (AG) as a compound that inhibits inducible nitric oxide synthase (iNOS) reduces ischemic injuries in various animal model of stroke (Zhang *et al*., 1996; Cash *et al*., 2001). These studies mainly have demonstrated that AG has neuroprotective effects when given in chronic phase of cerebral ischemia (Zhang and Iadecola, 1998; Nagayama *et al*., 1998; Takizawa *et al*., 1999; Sugimoto and Iadecola, 2002). For example, it has been reported that administration of AG between 24 and 96 h after ischemia attenuate post-ischemic iNOS activity and reduces size of infarct volume (Nagayama *et al*., 1998, Zhang *et al*., 1996). Moreover, very few studies have shown that AG significantly reduces brain damage in acute phase of permanent (Cockroft *et al*., 1996) or transient model of focal cerebral ischemia (Cash *et al*., 2001). Danielisova and colleagues reported that administration of AG immediately after ischemia significantly reduces cell damage in global model of cerebral ischemia (Danielisova *et al*., 2004). As well, our pervious study demonstrated that single dose of AG that was given one hour after middle cerebral artery occlusion (MCAO), significantly reduce brain ischemic injuries (Vakili *et al*., 2006). Most of these studies have focused on infarct volume and few studies exist about effect of AG on motor neurological dysfunctions. In addition, less experimental evidence is existing regarding to effects of AG on striatal and cortical infarct size in acute phase of focal cerebral ischemia. Therefore, present study was performed to evaluate effect of various doses of AG on infarct size and motor neurological dysfunction in a rat model of transient focal cerebral ischemia.

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MATERIALS AND METHODS

Animals

Male Wistar rats (Pastor Institute, Tehran, Iran) were housed in standard cages in a temperature (22-24 ºC), humidity (40-60%), and light period (07.00-19.00 h) - controlled environment. Experiments were performed in conformity with the university research council guidelines for conducting animal studies.

Transient focal cerebral ischemia

Middle cerebral artery occlusion was induced by intraluminal filament method as described previously (Vakili and Zahedi Khorasani, 2007). Briefly, animals were anesthetized with chloral hydrate (400 mg/kg ip) and the right common carotid (CCA) and external carotid artery (ECA) was exposed. A nylon thread (3-0) was carefully inserted into the internal carotid artery (ICA) and advanced towards the origin of the middle cerebral artery until a light resistance was felt. Such resistance was indication that tip of nylon thread was wedged at the beginning of anterior cerebral artery (20-22 mm from CCA bifurcation), resulting in occlusion of MCAO (15). After 60 minute of MCAO, reperfusion was accomplished by withdrawing the intraluminal filament. Animals were then recovered from anesthesia, and kept in single cages for 24 hrs. Rectal temperature was measured by a thermometer and maintained at 37 ± 0.5 ºC throughout the experiment using an electrical blanket.

Experimental design and protocol

Infarct volume was investigated 24 h after MCAO in four different group's rat. Group 1 (n=8) was control group which received saline as the vehicle (1ml/kg) at the beginning of MCAO. Groups 2-4 (each n=8) were treatment group which received AG (Sigma, Germany) at doses 75(n=8), 150(n=8) and 300(n=8) mg/kg ip at the beginning of MCAO, respectively.

Neurological evaluation

Neurological examination were performed, 24 hrs after MCAO blindly using five point scoring system as described previously (Vakili *et al*., 2007). Accordingly the scoring are as follows; 0 = normal motor function, 1 = flexion of contralateral torso or forelimb upon lifting by tail or failure to extend forepaw when suspend vertically, 2 = circling to the contralateral side but have normal posture at rest, 3 = loss of righting reflex, and 4 = no spontaneous motor activity.

Measurement of infarct volume

After neurological deficit was tested, animals were deeply anesthetized and sacrified by cervical dislocation. Subsequently, brains were removed and sectioned coronally into seven 2-mm thick slices using a Brain Matrix. Afterwards, slices were immersed in 2% Triphenyltetrazolium chloride solution (Sigma, Germany), and kept at 37^{o}C in a water bath for 15 minutes. The slices were then transferred to 10% buffered formalin (Merck, Germany). Twenty-four hours later, slices were photographed using a digital camera connected to a computer (Cannon-Japan). Infarct areas were first measured using an Image Analyzer Software (NIH Image Analyzer). The infarct volume of each slice was calculated by multiplying the infarct area of the slice by its thickness. The total infarct volume of each brain was calculated as the sum of the infarct volumes of the seven brain slices. The contribution of edema to the infarct volume was corrected by the following formula as previously described (Swanson *et al*., 1990, Vakili *et al*, 2005). Corrected infarct volume =Left hemisphere size - (Right hemisphere size -Measured infarct size).

STATISTICAL ANALYSIS

Data are presented as Mean ± SEM. For effect of AG on infarct volume, comparison of multiple groups were performed using one way Analysis of Variance (ANOVA) followed by Tukey test. For the effect of drug on neurological deficit score and infarct area in each sections of brain slice, comparison of multiple groups were performed by non parametric Kruskal-Wallis One Way Analysis of Variance on Ranks followed by a Dunnett's test (SigmaStat 2.0, Jandel Scientific, Erkrath, Germany). Differences were considered significant at P<0.05.

RESULTS

Dose dependent effect of AG on ischemic reperfusion injuries

Treatment with AG at doses 75, 150 and 300 mg/kg ip that administered at the beginning of MCAO significantly reduces total infarct volume (figs. 1 and 2, p<0.001). Moreover, there was significant difference between infarct volume from AG treated at doses 150 and 300 mg/kg (p<0.01). Administration AG at doses 75, 150 and 300mg/kg ip at the beginning of MCAO significantly reduces cortical infarct volume to 86±12, 61±9 and 82±10 *vs.*161±11 (P<0.001), respectively (fig 1). Furthermore, AG at dose 300 mg/kg did not alter striatal injuries (53±4 *vs.* 52±5, P>0.05), while at doses 75 or, 150 mg/kg significantly reduces striatal infarct volumes in comparison control group (31±5 and 32±4 *vs.* 52±5, p<0.01) respectively (fig 1). In addition, AG at dose 150 mg/kg ip significantly reduces infarct area in 1-6 sections, while AG at doses 75 to 300 mg/kg ip significantly reduces infarct area only in sections 3-5 in comparison saline (fig. 3).

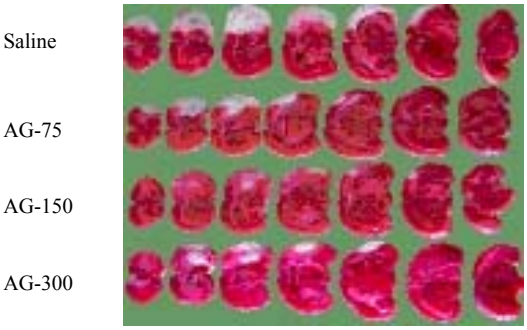

Fig. 1: Photographs illustrating the in seven coronal sections of rat brain with TTC staining, after 60 minute MCAO and 23 hours reperfusion, in which red color is normal area and white color is infarct area. Colorless region corresponds to occluded MCA territory.

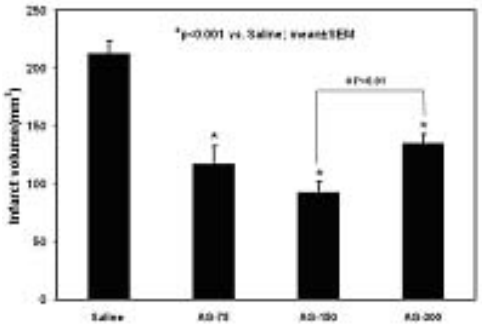

Fig. 2: Total infarct volume in saline and AG treated at doses 75 (AG-75), 150 (AG-150) and 300 (AG-300) mg/kg ip (n= 8 each). AG was given at the beginning of MCAO.

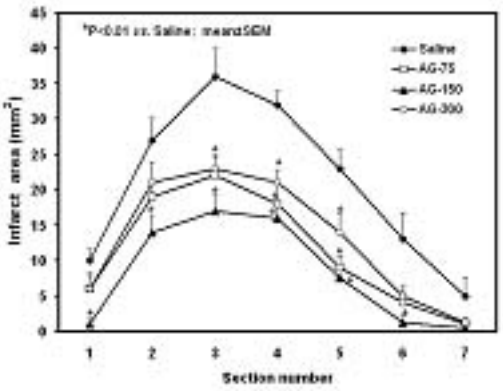

Fig. 3: Infarct areas for 7 coronal section form anterior to posterior in saline and AG treated at doses 75 (AG-75), 150 (AG-150) and 300 (AG-300) mg/kg ip (n= 8 each). AG was given at the beginning of MCAO.

Effect of AG on motor of neurological deficits score

Administration of AG only at dose 150 mg/kg resulted in a significantly improved motor neurological deficit score 24 hours after induction of cerebral ischemia (1.38 ± 0.18 *vs.* 2.12 ± 0.13) in comparison with saline group (P<0.01).While, AG at doses 75 and 300 mg/kg did not significantly change motor neurological dysfunction (P>0.05, fig. 4).

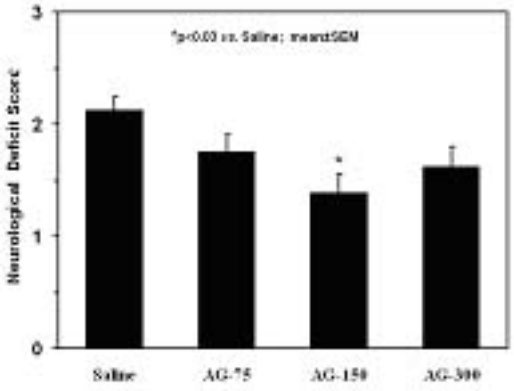

Fig. 4: Effect of treatment with saline or AG at doses 75 (AG-75), 150 (AG-150) and 300 (AG-300) mg/kg ip (n= 8 each) on neurological deficit score at 60 minute MCAO and 23 h after reperfusion.

DISCUSSION

We have shown that administration of AG reduces the size of infarct volume dose-dependently in acute phase of transient focal cerebral ischemia. Our results demonstrated that treatment with AG at dose 150 mg/kg ip, starting at the beginning of ischemia, significantly reduces cortical and striatal infarct volume. While, higher dose of AG (300 mg/kg) reduced the size of ischemic lesion in cerebral cortex but had no protective effect on striatum damage. This finding is similar to pervious observation that demonstrated treatment with AG at dose 300 mg/kg only decreases cortical damage without any effect on striatum damage in transient model of focal cerebral ischemia (Zhang *et al*., 1996). Moreover, it has been reported that pretreatment or early post-ischemic treatment with AG at dose 160 mg/kg effectively decrease infarct size in a permanent model of focal cerebral ischemia (Cockroft *et al*., 1996; Zimmerman *et al*., 1995) that support finding of present study. Taken together, our results suggest that AG is less effective in higher dose and might even aggravate post ischemic damage. One possible explanation for this finding is that AG at high dose might inhibit endothelial NOS (eNOS) (Laszlo *et al*., 1995). Nitric oxide produced by eNOS is beneficial in the early stage of cerebral ischemia, probably, by producing vasodilatation and by inhibiting platelet aggregation and leukocyte adhesion (Huang *et al*., 1996). Therefore, eNOS inhibition by AG may lead to further flow decrease to ischemic area and counteract protective effect of AG on striatum damage. Furthermore, present study is also showing that AG mediated neuroprotection is mainly seen in the posterior part of the MCA territory where cortical, that is, penumbral. This finding is in agreement with those of pervious studies demonstrating that neuroprotective agents are less effective or even ineffective in striatum (Plesnila *et al*., 2004; Vakili and Zahedi Khorasani, 2007).

Several studies indicated that iNOS might be involved in development of post-ischemic injuries (Nagayama *et al*., 1998; Iadecola *et al*., 1995a). However, it is unlikely that the decrease in infarct size produced by AG is related to iNOS inhibition in this study. Because, it has demonstrated that iNOS expression does not increase during the first 24 h after focal cerebral ischemia (Cash *et al*., 2001; Iadecola *et al*., 1995b). Whereas in this study, we administered AG at the beginning ischemia and measured brain infarct volume 24 h after ischemia, before iNOS expression increased. Thus, neuroprotective effect of AG observed in present study is likely related to effect of AG other than iNOS. It has been demonstrated that AG is a free radical scavenger against hydroxyl radicals, superoxide anion *in vitro* and an antioxidant in vivo (Yildiz *et al*., 1998; Giardino *et al*., 1998). It has reported that, free radicals and oxidants have important role in development and expansion ischemic injuries (Zhang and Ellis, 1990; Nelson *et al*., 1992; Chan, 1994; Kondo *et al*., 1997). Moreover, the neuroprotection exerted by AG can not related to its effects on cerebral blood flow, because previous study has demonstrated that AG does not affect on cerebral blood flow (Iadecola *et al*., 1995b). Likewise, protective effect of AG, can not related to changes in arterial blood pressure, blood gases and rectal temperature, because these parameters carefully monitored and did not significantly differ among groups, that is in agreement with other research (Zhang and Iadecola, 1998). Another potential mechanism by which AG could

decrease ischemic damage is by inhibition of advanced glycation end pro-product (AGE) production or cross-linking (Brownlee *et al*., 1986). Pr-ischemic administration of AGE exacerbates ischemic damage, an effect that is blocked by AG (Zimmerman *et al*., 1995).

In addition, our study demonstrated that administration of AG only at dose 150 mg/kg significantly improves neurological deficit after 60 min MCAO and 24 h reperfusion. Most importantly, the present study showed that the decrease in histology damage is reflected some improvement of the neurological deficits that associated with MCA occlusion. This finding also confirms neuroprotective of AG as a potential treatment for the early stages of cerebral ischemia. Although, there is no information about the early treatment of AG on the neurological deficits but our data are consistent with other finding showing delayed treatment with AG, 24 hours after ischemia, significantly improved neurological deficits as well as decreased cerebral infarct volume under permanent MCA occlusion (Nagayama *et al*.,1998). AG at doses 75 or, 300 mg/kg ip did not significantly change neurological deficits scores. It is possible that the reduction of ischemic injuries is not sufficient to improve neurological function. This hypothesis received support as the study have reported that large reduction in infarct sizes are necessary to improve neurological deficits score (Barone *et al*., 1997, Vakili and Zahedi Khorasani, 2007).

In conclusion, our findings show that AG decrease ischemic brain damage dose-dependently and improve neurological recovery in acute phase of transient focal cerebral ischemia. Moreover, we suggested AG at middle dose have the best neuroprotective activity and less effective in higher dose.

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