Experimental spinal cord ischemia: detection and protection

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Chapter 8

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Abstract

Background: Inhibition of neurotoxic events that lead to delayed cellular damage might prevent motor function loss after transient spinal cord ischemia. An important effect of the neuroprotective substance aminoguanidine (AG) is the inhibition of inducible nitric oxide synthase (iNOS), a perpetrator of focal ischemic damage. We have studied the protective effects of AG on hind limb motor function and histopathological outcome in an experimental model for spinal cord ischemia, and related these findings to the protein content of iNOS in the spinal cord.

Methods: Temporary spinal cord ischemia was induced by 28 min of infrarenal balloon occlusion of the aorta in forty anesthetized New Zealand white rabbits. Animals were randomly assigned to two treatments: saline (n = 20) or AG (n = 20, 100 $mg.kg^{-1}$ intravenously [i.v.] before occlusion). Postoperatively, treatment was continued with subcutaneous [s.c.] injections twice daily (saline or AG 100 $mg.kg^{-1}$). Normothermia (38 °C) was maintained during ischemia and rectal temperature was assessed before and after s.c. injections. Animals were observed for 96 h for neurologic evaluation (Tarlov score), and the lumbosacral spinal cord was examined for ischemic damage after perfusion-fixation. Finally, iNOS protein content was determined using Western blot analysis 48 h after ischemia in 5 animals of each group.

Results: Neurologic outcome at 96 h after reperfusion was the same in both groups. The incidence of paraplegia was 67% in the saline treated group versus 53% in the AG treated group. No differences in infarction volumes, total number of viable motoneurons, or total number of eosinophilic neurons were present between the groups. At 48 h after reperfusion iNOS protein content in the spinal cord was increased in one animal in the AG treated group and in three animals in the control group.

Conclusion: The data indicate that periischemic treatment with high dose aminoguanidine in rabbits offers no protection against a period of normothermic spinal cord ischemia. There was no conclusive evidence of spinal cord iNOS inhibition following treatment with aminoguanidine.

Introduction

Spinal cord injury is among the most disabling complications following aneurysm surgery of the thoracoabdominal aorta. Presumably, a prolonged period of spinal cord blood flow reduction results in a dynamic and extended activation of cytotoxic biochemical processes that ultimately results in neuronal cell death and function loss. In experimental cerebral ischemia aminoguanidine (AG) has been shown to improve neurologic injury, both with early and delayed administration.[1-3]

Neuroprotective effects by AG might be mediated by diverse mechanisms of action. Aminoguanidine is a modestly selective inhibitor of the inducible nitric oxide synthase (iNOS) isoform.[4] Excessive production of nitric oxide resulting from upregulation of iNOS has been thought to play an important role in the extension of neuronal injury during the reperfusion period following the ischemic insult.[2,5,6] In addition to iNOS inhibition, AG also modulates at least two other enzymatic systems that are associated with ischemia. Inhibition of the potentially damaging polyamine oxidase enzyme system and reduction of advanced glycation endproducts by AG might decrease ischemic neuronal damage in the early reperfusion phase.[1,7-9] It is presently unknown if the neuroprotective effects of AG, as reported in cerebral ischemia models, can prevent neuronal damage following transient spinal cord ischemia.

In the present study we have investigated the effects of periischemic AG treatment on neurologic and histopathologic outcome in a rabbit model of transient spinal cord ischemia. To investigate the role of AG-induced iNOS modulation during spinal cord ischemia, we additionally measured iNOS protein content in the spinal cord with and without AG treatment.

Materials and Methods

Animal care and all procedures were performed in compliance with the national guidelines for care of laboratory animals in the Netherlands. The study protocol was approved by the Animal Research Committee of the Academic Hospital at the University of Amsterdam, the Netherlands. Forty New Zealand White rabbits weighing 3.5 ± 0.2 kg were used in this study.

Anesthesia and monitoring

Animals were premedicated with ketamine (50mg.kg^{-1} intramuscular (i.m.)) and xylazine (10mg.kg^{-1}, i.m.). After intubation of the trachea anesthesia was maintained with a combination of isoflurane (1.5%) in a mixture of 50% O_2 in N_2O by mask and intravenous sufentanil (5µg. kg^{-1}.min^{-1}). Cefamandol (100mg) was given before incision. End-tidal CO_2 was measured by a mainstream capnograph (Hewlett-Packard) and P_aCO_2 was maintained within 35-40 mmHg. The initial tidal volume was 20 ml.kg^{-1} and the respiratory rate 40.min^{-1}. Rectal and paraspinal temperatures were monitored and kept at 38°C by means of a heating lamp. Paraspinal muscle temperature was measured using a needle probe (Subcutaneous Temperature Sensor, Monatherm Inc., St.Louis, USA), connected to a Mon-a-therm, model 6510 (Mallinkrodt Medical, Inc., St. Louis, USA). The electrocardiogram was recorded with paediatric surface electrodes and the mean arterial blood pressure was measured with a double lumen wedge catheter placed in the abdominal aorta. Before, during and after aortic occlusion arterial blood samples (1 ml) were drawn for measurement of blood gases, glucose and hematocrit. Just before awaking all animals received buprenorphine 0.05 mg.kg^{-1} subcutaneously for analgesia in the early postoperative period.

Operative technique

Under sterile conditions, a right femoral arteriotomy was performed 3 - 4 cm distal to the inguinal ligament. A 5Fr. double lumen wedge pressure balloon catheter (AI 07025, Arrow Holland) was advanced 14 cm into the femoral artery. In a previous study we confirmed by performing a laparotomy that this resulted in a balloon location 0.5 - 1.5 cm distal to the left renal artery in the abdominal aorta.[10] Before catheter insertion heparin 500 I.U. was administered (i.v.) followed by 500 I.U. every 30 min thereafter until catheter removal. The balloon was inflated until loss of pulsatile distal aortic pressure (as measured at the distal orifice of the catheter). The duration of aortic occlusion was 28 min, based on a previous study in which a 93% paraplegia rate was observed in isoflurane anesthetized animals.[11] After catheter removal the wound was closed and animals were allowed to recover. The period between reperfusion and extubation was kept constant to account for a possible additional neuroprotective effect of longer duration of anesthesia in the animals treated with the studied agent.

Drug administration

In a pilot study the maximum tolerable dose and the route of administration was determined for AG. An initial dosage of AG 300 $mg.kg^{-1}$ was based on studies in the rat.[1,3] This dose caused severe hypotension and bradycardia when given intravenously to rabbits (n = 6) before aortic occlusion. The maximum tolerable dose for rabbits under general anesthesia was AG 100 $mg.kg^{-1}$, and the optimal route of administration in the postoperative period was the subcutaneous route. In a separate group of animals (n = 6) intraperitoneal administration of the substance clearly caused sedation, which interfered with motor function scoring. Subcutaneous AG 100 $mg.kg^{-1}$ did not cause sedation.

Animals were randomly assigned to one of two treatment groups: control (C, n=20) and aminoguanidine ((AG), n=20). Aminoguanidine was supplied by Sigma (St.Louis, Missouri, USA). Fifteen minutes prior to aortic occlusion the animals received an i.v. injection of saline (C) or AG 100 $mg.kg^{-1}$ (AG). After emergence from anesthesia the animals received saline (controls) or AG 100 $mg.kg^{-1}$ s.c. twice daily for four days following the ischemic episode. Rectal temperature was measured just before and one hour after each injection.

Neurologic and histopathological evaluation

At 24, 48, 72 and 96 h after the ischemic insult, the neurologic status of the animals' hind limbs was assessed by an observer unaware of the treatment allocation, according to a modified Tarlov score (5-points grading scale)[12]: 0 = paraplegic with no lower-extremity function, 1 = poor lower-extremity function, weak anti-gravity movement only, 2 = some lower-extremity motor function with good anti-gravity strength but inability to draw legs under body and/or hop, 3 = ability to draw legs under body and hop but not normally, 4 = normal motor function. In paraplegic animals bladder contents were expressed manually when required.

Spinal cord pathology

After final scoring of neurologic function at 96 h, the animals were anesthetized with ketamine (50 $mg.kg^{-1}$ intramuscularly (i.m.)), xylazine (10 $mg.kg^{-1}$, i.m.) and isoflurane (1 MAC) in a mixture of 50% O_2 in N_2O. After administration of heparin (2500 I.U.), animals were killed with pentobarbital (100 mg, i.v.), and perfusion fixated with formalin 3.6%. Lumbosacral spinal cord was removed *en bloc* and immersed in formalin for at least 10 days.

The whole lumbosacral portion of the spinal cord was sampled systematically.[13] Twelve equidistant transverse slices (1 mm thick) were dissected and embedded in paraffin. From each paraffin block, randomly selected 4-μm thick sections were cut and stained with hematoxylin and eosin. One section from each block was evaluated by an observer blinded to the treatment condition as described below.

Infarction volume

At a low magnification all of the sections were digitised and the areas of 1) total gray matter and 2) infarcted gray matter were measured interactively using image analysis software (Qwin, Leica, Camebridge, U.K.). The areas (mm^2) were then integrated with the known distance between each transverse level to provide an estimate of the infarction volume of the spinal cord. In each animal, the extent of infarction was expressed as the percentage of necrotic tissue of the total gray matter volume. To further specify the localisation of infarctions, gray matter area was separated into dorsal, intermediate and ventral zones by dividing the dorsoventral axis of gray matter into three equal parts.

Selective neuronal necrosis

To quantify selective necrosis eosinophilic neurons were counted in every section of the spinal cord using light microscopy (Leica, Cambridge, U.K.). Individual counts were added to give an aggregate of eosinophilic neurons for all 12 sections. The effective magnification was 100x.

Ventral horn motoneurons

The total number of apparently viable ventral horn (α) motoneurons was determined in each section. Morphologic viability was defined according to criteria used in a previous study: fine granular cytoplasm with basophilic stippling (presence of Nissl substance), prominent nucleoli, and a soma diameter of 30-60 μm.[11] Results were expressed as aggregates of 12 counts for each animal, one count being the total number of motoneurons for one section.

Statistical analysis and presentation of results

Power-analysis was used to calculate the minimal group size that allowed for detection of significant differences in neurologic outcome between the treatment groups. We wished to have sufficient power ($1-\beta = 0.8$, $\alpha = 0.05$) to be able to detect a 50 % reduction of the paraplegia rate in the AG group, assuming a 85% event rate in the reference group; this requires a group size of 15.

Hemodynamic data, blood gases and temperatures are expressed as means ± standard deviation (SD). Tarlov scores are presented as medians and 10th to 90th percentiles. Infarction volumes, neuron counts and iNOS protein content are expressed as medians and interquartile ranges. The physiologic variables were analysed with a one-way analysis of variance and when significant differences were identified, student's t tests for intergroup comparisons with appropriate correction for multiple comparisons were carried out. Rectal temperature before and after s.c. injection was analyzed with ANOVA for repeated measurements. Comparison of the incidence of paraplegia (Tarlov = 0) was carried out using the Fisher exact test. To examine the correlation between neurologic function and infarction volumes, the Pearson's correlation coefficient was determined. Infarction volumes, neuron counts, Tarlov scores and iNOS protein content were analysed via the Kruskall-Wallis test followed by the Mann-Whitney U test when indicated. A P value of less than 0.05 was considered significant.

Western blot analysis of spinal cord iNOS content

Five animals treated with AG and five animals treated with saline (these animals were not part of the 96h survival group) were used to examine the iNOS protein content of the lumbosacral spinal cord level 4-5. After killing the animals with 300 mg nembutal at 48h after the ischemic insult the spinal cord was quickly harvested with the plunger of a 1 ml syringe and immediately frozen in liquid nitrogen. Inducible nitric oxide synthase content was determined semi-quantitatively with a Western blot analysis, using chemoluminescence.

The spinal cord samples were lysed with an UltraTurrax (Jahnke und Kunkel, Germany). The lysate was centrifuged (15000 g, 10 min) and the supernatant was frozen (-70°C). Lysate (10 µl) was mixed with 10 µl loading buffer (protein concentration 60 mg/ml) and was applied to a standard 10% SDS-page gel and electroblotted onto a polyvinylidene difluoride PVDF membrane. The blot was blocked with 5% casein-hydrolysate (Boehringer Mannheim, Germany) in phosphate buffered saline (PBS). The first antibody (anti-human

iNOS; NOS2 (C-19), sc-649, Santa Cruz Biotechnology, USA) was diluted 1:50 in 5% casein/PBS and incubated for 1 h at 30°C. The gel was washed (3 x 10 min) with PBS/0.05% NP 40. Thereafter the second antibody (goat-anti rabbit peroxidase-conjugated, Dako, Denmark) was administered (diluted 1:1000 in 5% casein/PBS) and incubated for 1 hr at 30°C. The gel was washed (3 x 10 min) and the chemolumiscent peroxidase substrate (LumiLight^{plus}, Roche Molecular Biochemicals) was added for 5 min. Thereafter quantification and detection was performed with the Lumi-Imager (Roche Molecular Biochemicals, Germany).

Results

Hemodynamic and blood gas data before, during and after aortic occlusion are summarized in table 1. No differences in MAP, heart rate, arterial oxygen tension, arterial carbon dioxide tension, hematocrit, or glucose were observed among the two groups. Normothermia (38.0 °C) was maintained during the operation, and there were no differences in observed temperatures between the groups. Postoperative rectal temperature before subcutaneous injection was the same in controls (39.2 ± 0.2 °C) and AG treated animals (39.2 ± 0.3 °C). Rectal temperature did not change 1 hr after injection in controls (39.0 ± 0.2 °C) and in AG treated animals (39.1 ± 0.4).

Ten animals were replaced during the course of the study. Two animals were sacrificed intraoperatively (one in group C and one in group AG, incomplete occlusion duration because of technical failure of the catheter resulting in premature balloon deflation). Eight animals were euthanized because of postoperative systemic complications. Two animals had pneumonia (both in group AG, Tarlov score = 4 and 0 respectively), one animal had peritonitis (group AG, Tarlov score not evaluated), three animals were euthanized because of stress associated with the neurologic deficit (one in group C and two in group AG, Tarlov = 0, 0 and not evaluated, respectively), and two animals died before 96 h post reperfusion without recognisable pathology during post mortem evaluation (both in group C, Tarlov scores both 0).

Table 1. Physiologic variables

Group	Proximal MAP, mmHg	Distal MAP, mmHg	HR, beats/min	PaO_2, mmHg	$PaCO_2$, mmHg	Hematocrit, %	Glucose, mmol/l
Control (n = 15)							
Preischemia	57 ± 12	-	170 ± 34	247 ± 40	35 ± 8	40 ± 3	7.5 ± 3.4
Ischemia	108 ± 38	8 ± 7	201 ± 33	227 ± 6	32 ± 2	38 ± 3	-
Reperfusion	58 ± 5	-	200 ± 26	280 ± 38	34 ± 4	40 ± 3	-
Aminoguanidine (n = 15)							
Preischemia	63 ± 12	-	197 ± 39	252 ± 63	32 ± 7	39 ± 3	8.0 ± 3.2
Ischemia	104 ± 29	10 ± 5	174 ± 32*	257 ± 48	35 ± 3	37 ± 3	-
Reperfusion	65 ± 9	-	185 ± 25	255 ± 75	34 ± 5	41 ± 3	-

All values are means ± SD. MAP = mean arterial pressure; HR = heart rate; Preischemia = period before occlusion; Ischemia = occlusion; Reperfusion = period after occlusion. * $P < 0.05$ as compared to controls.

Neurologic Outcome

The incidence of paraplegia and the average Tarlov scores at 24, 48, 72 and 96 h after reperfusion were the same for both treatment groups. (figure 1) The median Tarlov scores (+interquartile ranges) 96 h after the ischemic insult were 0 (0 – 3.8) in the control group and 1 (0.3 – 2.8) in the group treated with AG. Most of the neurologic deficit was present at 24 h after reperfusion and remained constant over time (24 – 96h). The incidence of paraplegia was 67% in the saline treated group versus 53% in the AG treated group (fig. 1).

Histopathology

In the animals with histologic injury, the infarctions typically affected the intermediate gray matter and the ventral horn gray matter (both 50% of gray matter total volume). The dorsal horn was relatively spared (33% infarction of gray matter total volume). The histopathologic results are summarised in table 2. The total number of viable motoneurons and eosinophilic neurons was the same in both groups (fig. 2, Table 2).

Figure 1 shows individual infarction volumes and neurologic function (Tarlov) scores for all animals at 96 h after reperfusion. There was a good correlation between Tarlov scores and infarction volumes (Spearman correlation coefficient 0.796, $P < 0.01$). Infarction volumes were not significantly different between the treatment groups. There was no difference in relative or absolute infarction volume between the treatment groups after subdivision of the total infarcted area into ventral, medial and dorsal zones.

Spinal cord iNOS protein content

No differences in intraoperative MAP, heart rate, arterial oxygen tension, arterial carbon dioxide tension, hematocrit, or glucose were observed between the group treated with saline and the group treated with AG (n = 5 each). There were no differences in paraspinal or rectal temperatures between the groups. At 48 h after reperfusion no difference in neurologic deficit was present between the two treatment groups. In figure 3 the iNOS content in the lumbosacral spinal cord is shown for each group as the raw data from the Lumi-Imager. At 48 h after reperfusion iNOS protein content in the spinal cord was increased in one animal in the AG treated group and in three animals in the control group. The median luminescence was 30183 in the control group (iqr: 7986 – 104116), and 6030 (4562 – 12087) in the AG group (P = 0.14). One animal (group AG) was not analysed because of insufficient material.

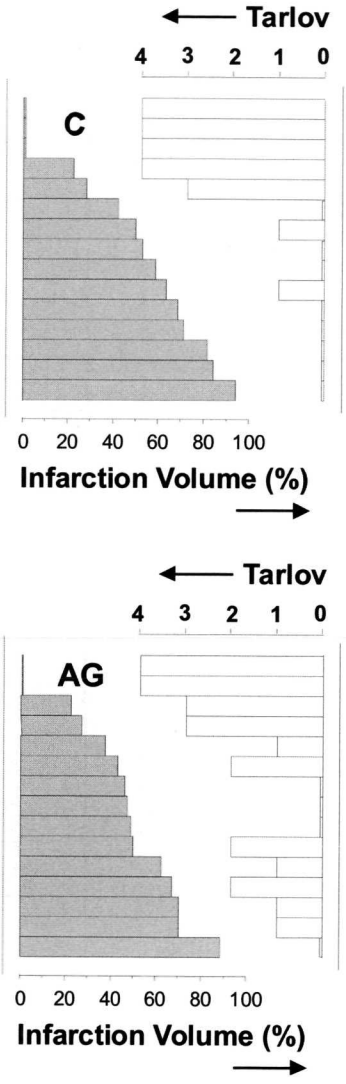

Figure 1:
Histograms of neurologic score (Tarlov) and relative infarction volume per individual animal in the two treatment groups. Infarction volume is shown at the left of each diagram (abscissa at bottom), neurologic score is shown at the right of each diagram (abscissa at top). Neurologic score: 0 = paraplegic with no lower-extremity function, 1 = poor lower-extremity function, weak anti-gravity movement only, 2 = some lower-extremity motor function with good anti-gravity strength but inability to draw legs under body and/or hop, 3 = ability to draw leg under body and hop but not normally, 4 = normal motor function. C = control; AG = aminoguanidine.

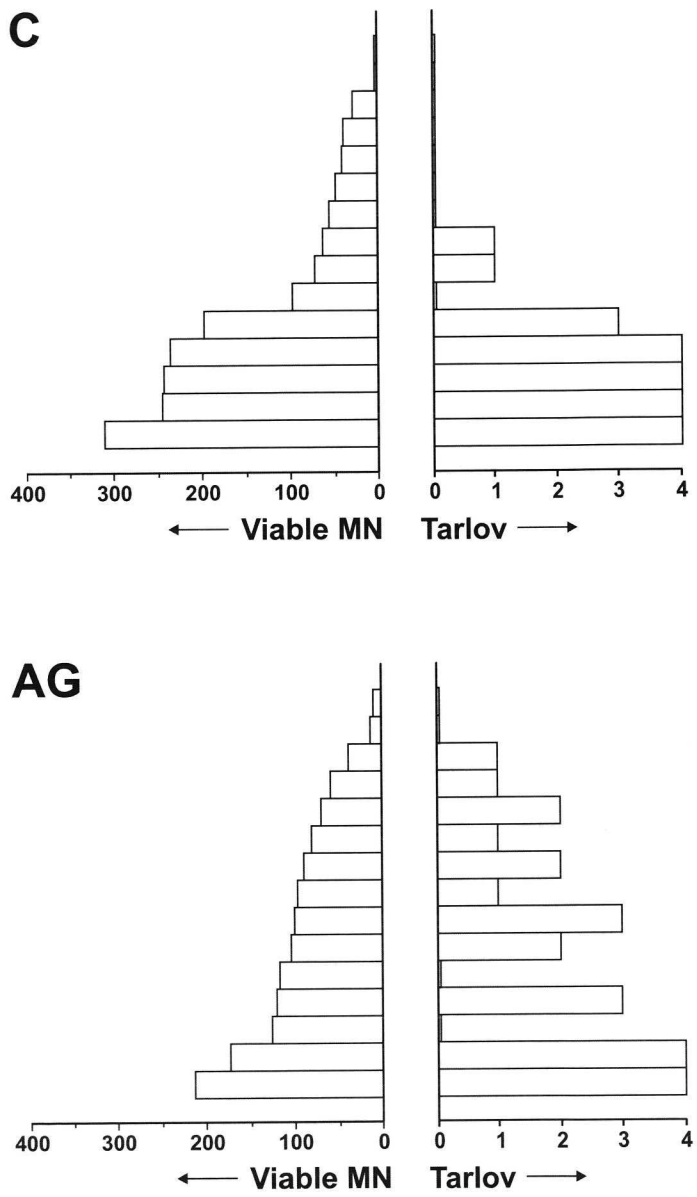

Figure 2:
Histograms of neurologic score (Tarlov) and the total number of viable motoneurons in the ventral horn per individual animal in the two treatment groups. The number of motoneurons is shown at the left of each diagram, neurologic score is shown at the right of each diagram. Neurologic score: 0 = paraplegic with no lower-extremity function, 1 = poor lower-extremity function, weak anti-gravity movement only, 2 = some lower-extremity motor function with good anti-gravity strength but inability to draw legs under body and/or hop, 3 = ability to draw leg under body and hop but not normally, 4 = normal motor function. C = control; AG = aminoguanidine. Viable MN = total number of viable motoneurons in the ventral horn. Tarlov = neurologic score.

Table 2. Infarction volumes and neuronal cell counts in the treatment groups

Group	Total gray	Total infarction	Infarction %	Viable motoneurons	Eosinophilic neurons
Control (n = 15)	216.7 (213.7 – 239.8)	127.9 (53.9 – 163.3)	53.1 (23.8 – 70.7)	62 (39 - 226)	13 (8 – 32)
Aminoguanidine (n = 15)	226.9 (211.9 – 245.8)	112.6 (64.0 – 154.4)	47.7 (29.7 – 65.9)	97 (61 - 120)	20 (3 – 37)

Data are expressed as medians + interquartile ranges. Total gray = total volume of gray matter (mm^3); Total Infarction = total volume of infarcted gray matter (mm^3); Infarction % = relative volume of infarcted gray matter.

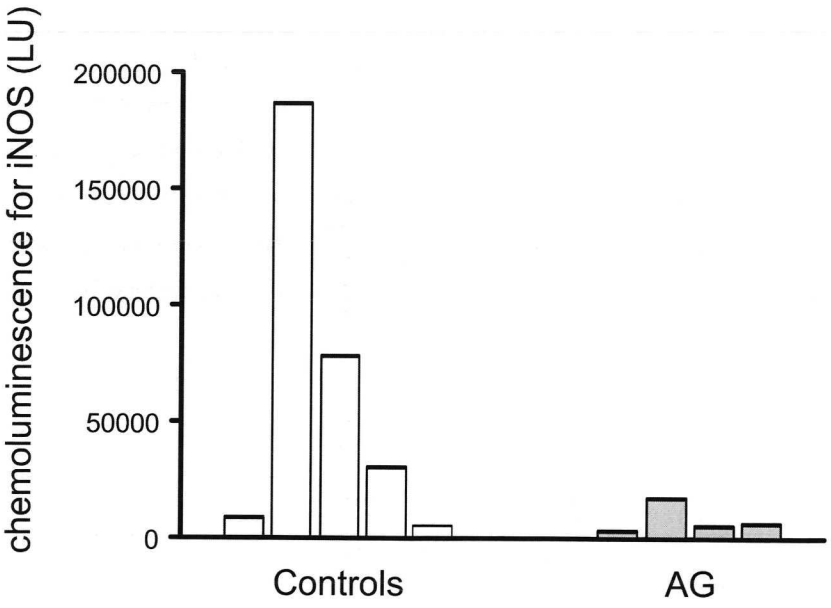

Figure 3:
The iNOS protein content at 48 hr post reperfusion as raw chemoluminescent data from the Lumi-Imager. The vertical axis represents chemoluminescence in light units (LU). Controls = animals (n = 5) treated with saline; AG = animals (n = 4) treated with aminoguanidine.

Discussion

In the present study, periischemic aminoguanidine (AG) did not improve neurologic and histopathologic outcome 96 hours after temporary spinal cord ischemia (SCI). These findings are in contrast to the results found with AG treatment in models of focal cerebral ischemia. Most experimental evidence for the cerebroprotective effects of AG treatment is related to the improvement of morphologic endpoints only, i.e. reduction of infarct size.[1,2,5,9] The one study in which neurologic function scores were reported after AG treatment, carried out by Nagayama and co-workers, showed less deficit between 48 and 96 hr in a small group of rats after permanent occlusion of the middle cerebral artery.[3]

We determined the maximum tolerable dose of AG in a pilot study based on doses that were protective in the rat.[1,3] Doses higher than 100 $mg.kg^{-1}$ might be toxic for rabbits, probably based on inhibition of diamine oxidase by AG [Personal communication: Dr Xian-Liang Tang, M.D.Assistant Professor, Cardiology Division, University of Louisville and Dr Michael Brownlee, M.D., Anita and Jack Saltz Professor of Diabetes Research, Dept. of Medicine and Pathology, Albert Einstein College of Medicine, New York]

Several mechanisms have been proposed for the neuroprotective effects of AG, each of which occurs at different time windows during the ischemic cell death process. Delayed administration of AG reduced infarction size in rats submitted to focal cerebral ischemia, which was attributed to inhibition of iNOS upregulation.[2,3,5] The presence of excessive concentrations of nitric oxide (NO) during the reperfusion phase of focal cerebral ischemia has been related to a worsened outcome in terms of infarct size, which can be improved by inhibition of nitric oxide synthases.[2,14] Especially activation of the iNOS isoform results in concentrations of NO that are toxic for surrounding neurons.[15] Other evidence from transgenic models indicates that mice that lack the gene for iNOS have smaller infarcts than wild-type controls after focal cerebral ischemia.[16]

Semi-quantitative determination of spinal cord iNOS protein content suggests that in AG treated animals 48 h after reperfusion iNOS upregulation is prevented. Moreover, two animals in the control group showed no iNOS upregulation (fig.3). However, because of the small sample size of the iNOS protein assay, the present data lack statistical power, and we cannot conclude that the maximum tolerable dose of AG induces consistent iNOS inhibition in rabbits. The possibility exists that in the current experimental design, timing of iNOS determination was not adequate for optimal detection of the treatment effect of AG. However,

this seems unlikely because Iadecola et al. showed that iNOS mRNA expression in rat peaked at 48 hr reperfusion after cerebral ischemia.[17] Thus, there appears to be some inhibition of the iNOS system in the present study, although the small number of animals weakens the power of statistical comparison.

Early treatment with single-dose AG (320 $mg.kg^{-1}$) decreased infarction volume 24 h after reperfusion in a rat model of focal cerebral ischemia.[1,9] The authors suggested that apart from inhibition of iNOS, the reduction of polyamine oxidase (PAO) activity might have played a role in the neuroprotective effects of AG. Enhanced PAO activity occurs after stroke and results in an increased putrescine level, a polyamine that is involved in augmentation of glutamatergic effects on N-Methyl-D-aspartate receptor function at the neuronal membrane.[18,19] Also, toxic by-products of PAO upregulation in the early reperfusion phase after stroke, such as aldehydes and hydrogen peroxide, are very damaging to neurons in the penumbral region.[20,21] Ivanova et al. showed that the reduction of cytotoxic 3-aminopropanal, which is a catabolic product of PAO, was associated with reduced cerebral infarction in AG treated animals.[9] The effect of AG on polyamine metabolism was not determined in the present study. However, to provide optimal neuroprotection both in the early and late reperfusion phase we opted to administer AG both before and after aortic occlusion in order to have sufficient circulating levels to block the PAO pathway and prevent iNOS upregulation.

In the current study, a lower than expected incidence of severe neurologic deficit (defined as the total number of animals with Tarlov 0 or 1) was observed in the control group, which reduced the power. While in a previous study 93% of the animals were paraplegic at 72 hr after aortic occlusion this was only 67% in the present study.[11] Pretreatment with ketamine and xylazin in stead of isoflurane might explain the relatively low number of paraplegic animals in the present control group because at least ketamine seems to have cerebroprotective properties.[22] However, ketamine did not provide neuroprotection in the present model of rabbit spinal cord ischemia.[11]

The possible insufficient inhibition of the iNOS enzyme system might have precluded the detection of neuroprotective effects of AG treatment in the present study. However, there are other possible reasons that might explain the difference in treatment effect compared to previous studies. Because the neuroprotective effect of periischemic AG was studied in a

rabbit model of transient spinal cord ischemia, the current findings might not be compatible with studies that tested AG in cerebral ischemia models. For example, biochemical and physiologic differences between spinal cord and the brain or interspecies differences might account for the observed differences in neurologic outcome after AG treatment.

Experimental Model

In the present study we produced spinal cord ischemia in the rabbit by infrarenal aortic occlusion. This is a highly reproducible model for the production of spinal cord ischemic injury, because this animal has a segmental blood supply to the spinal cord with poor collateral flow between the segments. In general, a clear relationship exists between the occlusion period, histopathologic damage, and clinical function.[23] This makes it a reliable model for assessment of putative neuroprotective pharmacological agents. Evaluation of the effects of periischemic AG consisted of neurologic function scoring, the quantification of global and selective neuronal necrosis, and the determination of the total number of viable motoneurons in the ventral horn. Thus, in the present study differences in outcome between the treatment groups were not detected both with functional measurements and extensive histopathologic estimation of neuronal damage. In addition, the possible toxic effects of high doses of AG in the awake rabbit may not justify further assessment of the neuroprotective effects of the substance in the present model.

Conclusion

The results of this study indicate that periischemic aminoguanidine fails to ameliorate both functional and histopathologic evidence of ischemic neuronal damage after transient spinal cord ischemia in the rabbit. There was no conclusive evidence of spinal cord iNOS inhibition following treatment with aminoguanidine.

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