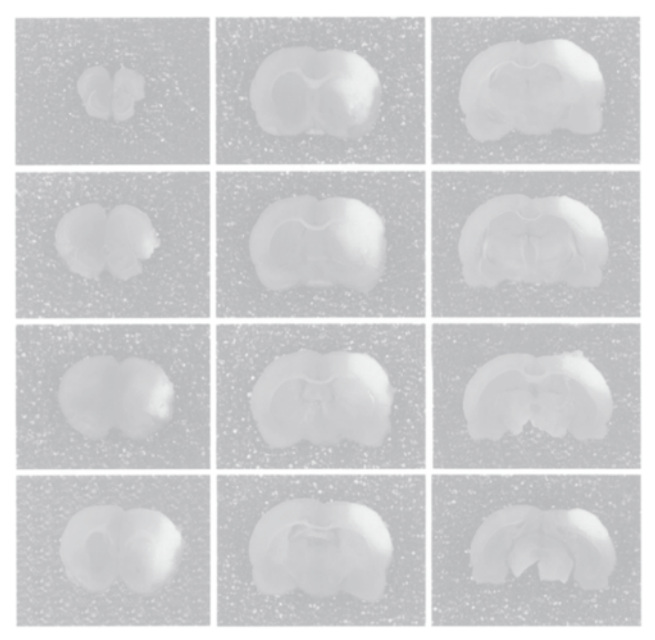
Nitric oxide in focal cerebral ischemia, an experimental study

Coert, B.A.

Publication date
2008

Chapter 4

Exogenous Spermine Reduces Ischemic Damage in a Model of Focal Cerebral Ischemia



Bert A. Coert MD
Robert E. Anderson BS
Fredric B. Meyer MD

Neuroscience Letters 282(2000) pp 5-8

ABSTRACT

Alterations in polyamine metabolism during and after global or focal cerebral ischemia can produce a multiplicity of effects on the brain, such as modification in the mitochondrial calcium buffering capacity, exacerbating glutamate-mediated neurotoxicity, and impairment of the blood-brain barrier. In this study, the endogenous polyamine spermine was administered intravenously 30 minutes prior to temporary focal cerebral ischemia in rats induced by clipping of the left middle cerebral and bilateral common carotid arteries for 3 hours. Three days after removal of the microclips, intracardiac perfusion with 2% 2,3,5-triphenyl tetrazolium chloride was performed. Coronal slices were cut, photographed, and examined for cortical infarct volume. Spermine reduced infarct volume in a dose-dependent fashion. This study demonstrates that the use of polyamines may be considered as a powerful tool in the prevention of ischemic tissue damage following focal cerebral ischemia.

INTRODUCTION

The polyamines (PA) putrescine, spermine, and spermidine are important for cell growth and differentiation[29]. They are involved with numerous cellular functions including protein phosphorylation and protein synthesis[17], regulation of gene expression[4], programmed cell death[9] , inhibition of membrane permeability transition of mitochondria[16], regulation of mitochondria Ca^{2+} transport[13,18], regulation of nitric oxide synthase (NOS)[12], and free radical scavenging[10,30]. Spermine in particular, found in millimolar concentrations in the nucleus of the cell[25] was shown to prevent endonuclease-mediated DNA fragmentation[3]. The role of polyamines in the pathophysiology of cerebral ischemia has not been very well elucidated. Following either transient global, focal, or focal permanent cerebral ischemia there is a significant increase in polyamine metabolism which is characterized by increased ornithine decarboxylase (ODC) activity with increased putrescine levels[21,23] which is subsequently metabolized to spermidine and spermine[29]. It has been demonstrated that spermine concentrations in brain is either slightly reduced [20,22,23] or moderately increased[15] following global cerebral ischemia. However, in models of focal cerebral ischemia, significant reductions[21,26] or no alterations[2] in Spermine concentrations have been demonstrated. It has been shown that in different models of ischemia, polyamine metabolism is dependent on changes in ATP and acetyl CoA[27]. Spermine has been reported to be neuroprotective in a model of forebrain ischemia. Gilad and Gilad[8], demonstrated in gerbils that intra-peritoneal administration of Spermine significantly decreased hippocampus and striatal cell loss. In a rat model of forebrain ischemia, Farbiszewski et al.[7], showed that spermine significantly reversed the decrease in superoxide dismutase (SOD) activity in the cortex. To date there have been no studies performed to ascertain the effects of endogenous spermine in a rat model of reversible focal cerebral ischemia. In this study we demonstrate that spermine can be significantly neuroprotective in this model.

MATERIALS AND METHODS

Following review and approval by the Institutional Animal Care and Use Committee, 24 adult male Wistar rats were administered halothane anesthesia in a mixture of oxygen and air through a face mask at 1.5% during the surgical procedure and 1.0% during the occlusion period. Subcutaneous glycopyrrolate (Robinul-V) was administered preoperatively at 4 mg/kg to reduce respiratory secretions. Core body temperature was continuously monitored at the beginning of the surgical preparation and throughout the experiment with a rectal probe. This rectal probe was connected to an infrared heating lamp, which maintained both the body and head temperature at 37.0 ± 0.5°C through-

out the experiment. Polyethylene catheters (PE-50) were inserted into the right femoral artery and vein for monitoring of mean arterial blood pressure (MABP) and arterial blood sampling (pHa, $PaCO_2$, PaO_2, hematocrit, serum glucose, and lactate). These physiological parameters were measured at the beginning of the surgical preparation, 15 min prior to ischemia, and at the completion of the ischemia experiment. The original technique as described by Tamura et al. [31], was modified for our experiments to increase the severity and reliability of the ischemic model[5]. A ventral midline incision was made for exposure of both common carotid arteries (CCA). The contralateral right CCA was permanently ligated using a 3/0 silk suture. The ipsilateral (left) CCA was temporarily occluded for 3 hours using a Mayfield micro-aneurysm clip. A skin incision was made between the left outer canthus and the tragus. The temporal muscle was deflected anteriorly and part of the left zygomatic arch was removed. Care was taken to avoid damaging the facial nerve. After anterior and downward retraction of the musculature, the mandibular nerve was identified and followed back to the foramen ovale. Using a high-speed air drill with irrigation, a 3-4 mm craniectomy was made just anterior and superior to the foramen ovale. The dura was opened with a sharp needle and the middle cerebral artery (MCA) was freed of arachnoid. The left MCA crossing the olfactory tract was temporarily occluded (3 h) using a Sundt #3 AVM microclip (Codman and Shurtleff Inc.).

Experimental groups

The 24 animals were divided into four groups as follows: spermine (Sigma) dissolved in NaCl 0.9 % was administered intravenously (i.v.) 30 min prior to middle cerebral artery occlusion (MCAo) at doses of 0.1 (n = 6), 1.0 (n = 6), and 10.0 (n = 6) mg/kg. An ischemic control group was included in which 0.35 ml of NaCl 0.9% i.v. (n = 6) was administered 30 min prior to MCA occlusion. Each solution was prepared directly before administration.

Histopathology

Three days (72 h) after removal of the left MCA and CCA clips, the animals were reanesthetized with pentobarbital and intracardially perfused with a warm (378 C) 2% TTC (2,3,5,-triphenyltetrazolium chloride) solution. Their brains were quickly removed, immersed in the 37ºC TTC solution for 15 min to enhance staining and then placed in 10% buffered formaldehyde for 5 days. Twelve serial coronal sections from each brain were cut at 1 mm intervals beginning at 3.7 mm from the bregma using a rodent brain matrix (ASI Instruments, Inc.) and the anterior side of each section photographed. Photographic slides were analyzed in a blinded fashion using a computer-assisted image analyzer (JAVA, Jandel Scientific Software). Total cortical infarct volume was calculated by integrating the infarcted area of all 12 sections (area of infarct in mm^2 x thickness of section).

Statistical Analysis

Statistical analysis was carried out using ANOVA with Tukey's post hoc test for multiple comparisons. Differences were considered significant if $P < 0.05$. The data is depicted as mean and standard error.

RESULTS

There were no significant differences in the measurement of systemic parameters including $PaCO_2$ (40 ± 1 mmHg), PaO_2 (214 ± 9 mmHg), pHa (7.434 ± 0.016), mean arterial blood pressure (96 ± 2 mmHg), and hematocrit (38 ± 1%) between the measurements taken prior to onset of ischemia and at the completion of the experiment and between all groups studied. Serum glucose decreased significantly ($P < 0.05$), but not in a dose-dependent manner, by an average of 30% after administration of spermine. Serum lactate decreased in a dose-dependent manner by 4, 27 and 48% after administration of 0.1, 1.0 and 10.0 mg/kg spermine, respectively. These values, however, were not significantly different from one to the other by ANOVA. Three hours of left MCA and bilateral CCA occlusion resulted in mean cortical infarct volume of 95.0 ± 7.4 mm^3 (Fig. 1). Intravenous administration of spermine 30 min prior to ischemia resulted in a dose-dependent response in the reduction of infarct volume (Fig. 1). A significant reduction ($P < 0.05$) of infarct volume was seen only in the 10 mg/kg spermine treated group compared to the control group (31.1 ± 17.4 vs. 95.0 ± 7.4 mm^3).

DISCUSSION

For the first time in a rat model of reversible focal cerebral ischemia, the findings of this study demonstrate an impressive and significant reduction of cortical infarct volume when spermine, an aliphatic polyamine, was administered intravenously 30 min prior to middle cerebral and common carotid artery cerebral occlusion. It also demonstrated no adverse effects on blood pressure, temperature, or blood glucose levels during the experimental period. Spermine is fully protonated at physiological pH and is readily solublized in organic and aqueous media. It has been shown that spermine can easily pass the blood-brain barrier [8,11,24]. Many early studies have shown increases, decreases, or no change in spermine concentrations in brain during and/or after reperfusion. It has shown that spermine is slightly reduced following global cerebral ischemia [20,22,23], while Koenig et al.[15], reported modest increase in tissue levels of spermine. In a rat model of focal cerebral ischemia by reversible middle cerebral artery embolization, Paschen et al.[21], reported a significant (≈23%) reduction in cortical spermine concentration during

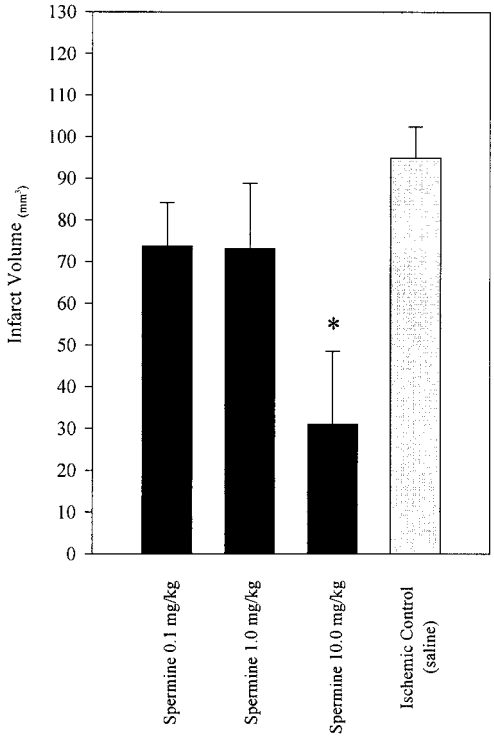


Figure. 1. Graph depicting cortical infarction volume in the ischemic control group (grey bar) and the spermine-treated groups (black bars) at 0.1, 1.0 and 10.0 mg/kg. Note that spermine was significantly (*P < 0:05, ANOVA) effective at the 10.0 mg/kg dosage.

ischemia. Most notably, spermine levels in both the contralateral and ipsilateral cortex after 24 h of reflow were less than the level seen in the ipsilateral cortex during ischemia. Sauer et al.[26], in a model of permanent focal brain ischemia, demonstrated a significant <50% reduction in spermine levels.

Baskaya et al.[2], using a model of focal cerebral ischemia in the cat, found no differences in spermine levels between ipsilateral ischemic cortex and penumbra compared to that of the contralateral side. Spermine synthesis involves S-adenosylmethioine derived from ATP and interconversion via acetylation steps involving acetyl-CoA. Therefore, in different models of ischemia, polyamine metabolism is dependent on changes in ATP and acetyl-CoA [27]. Many different mechanisms have been proposed to explain the protective and neurotoxic effects of polyamines. Blood-brain barrier (BBB) disruption was reported with the suggestion that the hyperosmolar (mannitol) BBB disruption could be mediated by increased ODC activity with significantly increased putrescine levels and slightly increased levels of spermine and spermidine[14]. N-methyl-D-aspartate (NMDA) receptor modulation at a specific site has also been proposed with a biphasic response with lower

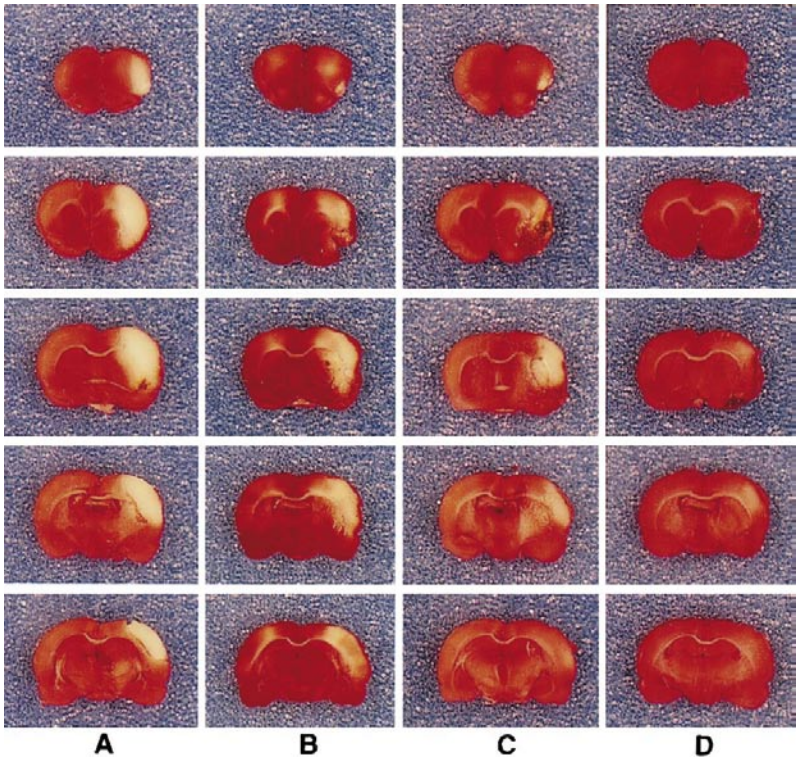


Figure. 2. Typical photographs of histological assessment using TTC staining after 3 h of focal cerebral ischemia followed by 72 h of reperfusion: (A) ischemic control animal; (B) 0.1 mg/kg spermine treated animal; (C) 1.0 mg/kg spermine treated animal; (D) 10.0 mg/kg spermine treated animal. Sections are at 2 mm intervals in descending order (anterior to posterior) beginning at the top row 3.7 mm from the bregma.

doses of spermine (<50 nmol) attenuating NMDA damage while higher dosages increased excitotoxic damage[19]. Separate recognition sites were identified for polyamines on the NMDA receptor, the presence of both positive and negative modulation implying more than one site[19]. Spermine and, to a lesser extent, spermidine were found to increase rate and affinity of Ca^{2+} uptake by mitochondria. This effect was progressively less potent with increasing Ca^{2+} concentration. Polyamines may play a critical role in the regulation of the intracellular Ca^{2+} concentration especially during activation[18]. Hu et al.[12] demonstrated an interaction between the positively charged amine groups in PA and the NADPH electron transfer of nNOS resulting in an inhibition of activity. Farbiszewski et al.[7], demonstrated a protective effect of exogenous spermine as an antioxidant enzyme defense in an in vivo rat model. Thiobarbituric acid-reactive substance (TBARS), an indicator of lipid peroxidase was diminished in this study by the addition of spermine (5 mg/kg i.v.) [7]. Interconversion enzyme activity SAMD (S-adenosyl methionine decarboxylase) and SSAT (spermidine/spermine acetyl transferase) regulate the interconversion from putrescine

to spermidine to spermine and vice versa. Spermine has been shown to be protective in neurotoxic insult of brain neurons in culture[1]. Spermine, given intra-peritoneally, significantly reduced hippocampal and striatal cell loss after an ischemic insult[8]. Many of the protective effects of PA attributed to spermine, while putrescine levels were reported to correlate well with the extent of tissue damage[20]. By changing the ratio of production of the different polyamines, SSAT activity at critical post-lesion periods may be relevant in determining the effect of PA[6]. Recently the polyamine oxidase activity in a traumatic brain model was found to contribute to edema formation and necrotic cavitation[6]. The harmful effects were found to be the production of putrescine, hydrogen peroxide and a toxic aldehyde [28]. Intravenously administered exogenous spermine in our model resulted in a significant reduction of infarct size. Further investigations are required to confirm this protective effect and to further ascertain the mechanism of action.

REFERENCES

1. Abe, K., Nishiyama, N. and Saito, H., Spermine promotes the survival of primary cultured brain neurons. Brain Res., 605 (1993) 322-326.
2. Baskaya, M.F., Rao, A.M., Dogan, A., Donaldson, D., Gellin, G. and Dempsey, R.J., Regional brain polyamine levels in permanent focal cerebral ischemia. Brain Res., 744 (1997) 302-308.
3. Brüne, B., Hartzell, P., Nicotera, P. and Orrenius, S., Spermine prevents endonuclease activation and apoptosis in thymocytes. Exp. Cell. Res., 195 (1991) 323-329.
4. Celano, P., Baylin, S.B. and Casero Jr., R.A., Polyamines differentially modulate the transcription of growth-associated genes in human colon carcinoma cells. J. Biol.Chem., 264 (1989) 8922-8927.
5. Coert, B.A., Anderson, R.E. and Meyer, F.B., Reproducibility of cerebral cortical infarction in the Wistar rat following middle cerebral artery occlusion. J. Stroke Cerebrovasc.Dis., Vol.8, No 6 (1999): pp380-287.
6. Dogan, A., Rao, A.M., Baskaya, M.K., Hatcher, J., Temiz, C.,Rao, V.L.R. and Dempsey, R.J., Contribution of polyamine oxidase to brain injury after trauma. J. Neurosurg., 90(1999) 1078-1082.
7. Farbiszewski, R., Bielawski, K., Bielawska, A. and Sobaniec,W., Spermine protects in vivo the antioxidant enzymes in transiently hypoperfused rat brain. Acta Neurobiol. Exp., 55(1995) 253-258.
8. Gilad, G.M. and Gilad, V.H., Polyamines can protect against ischemia-induced nerve cell death in gerbil forebrain. Exp.Neurol., 111 (1991) 349-355.
9. Ha, H.C., Woster, P.M., Yager, J.D. and Casero Jr., R.A., The role polyamines catabolism in polyamine analogue induced programmed cell death. Proc. Natl. Acad. Sci.USA, 94 (1997) 11557-11562.
10. Ha, H.C., Sirisoma, N.S., Kuppisamy, P., Zweier, J.L.,Woster, P.M. and Casero Jr., R.A., The natural polyamine spermine functions directly as a free radical scavenger.Proc. Natl. Acad. Sci. USA, 95 (1998) 11140-11145.
11. Halliday, C.A. and Shaw, G.G., Clearance of the polyamines from the perfused cerebroventricular system of the rabbit. J. Neurochem., 30 (1978) 807-812.
12. Hu, J., Mahmoud, M.I. and El-Fakahany, E.E., Polyamines inhibit nitric oxide synthase in rat cerebellum. Neurosci.Lett., 175 (1994) 41-45.
13. Jensen, J.R., Lynch, G. and Baudry, M., Polyamines stimulate mitochondrial calcium transport in rat brain. J. Neurochem.,48 (1987) 765-772.
14. Koenig, H., Goldstone, A.D. and Lu, C.Y., Polyamines mediate the reversible opening of the blood-brain barrier by the intracarotid infusion of hyperosmolal mannitol. Brain Res., 483 (1989) 110-116.
15. Koenig, H., Goldstone, A.D., Lu, C.Y. and Trout, J.J., Brain polyamines are controlled by N-methyl-D-aspartate receptors during ischaemia and recirculation. Stroke, 21 (1992)98-102.
16. Lapidus, R.G. and Sokolove, P.M., Inhibition by spermine of the inner membrane permeability transition of isolated rat heart mitochondria. FEBS Lett., 313 (1992) 314-318.
17. Lenzen, S., Hickethier, G. and Paten, U., Interactions between spermine and Mg21 on mitochondrial Ca21 transport. J. Biol. Chem., 261 (1986) 16478-16483.
18. Lenzen, S., MuÈ nster, W. and Rustenbeck, I., Dual effect of spermine on mitochondrial Ca21 transport. Biochem. J., 286 (1992) 597-602.
19. Munir, M., Subramamiam, S. and McGonigle, P., Polyamines modulate the neurotoxic effects of NMDA in vivo. Brain Res., 616 (1993) 163-170.

20. Paschen, W., Schmidt-Kastner, R., Hallmayer, J. and Djuricic, B., Polyamines in cerebral ischemia. Neurochem.Pathol., 9 (1988) 1-20.

21. Paschen, W., Csiba, L., Röhn, G. and Bereczki, D., Polyamine metabolism in transient focal ischemia of rat brain. BrainRes., 566 (1991) 354-357.

22. Paschen, W., Widmann, R. and Weber, C., Changes in regionalpolyamine profiles in rat brains after transient cerebral ischemia (single versus repetitive ischemia): evidence for release of polyamines from injured neurons. Neurosci.Lett., 135 (1992) 121-124.

23. Paschen, W., Polyamine metabolism in reversible cerebral ischaemia. Cerebrovasc. Brain Metab. Rev., 4 (1992) 59-88.

24. Pateman, A.J. and Shaw, G.G., The uptake of Spermidine and spermine by slices of mouse cerebral hemispheres. J.Neurochem., 25 (1975) 341-345.

25. Sarhan, S. and Seiler, N., On the subcellular localization of the polyamines. Biol. Chem. Hoppe-Seyler, 370 (1989)1279-1284.

26. Sauer, D., Martin, P., Allegrini, P.R., Bernasconi, R., Amacker, H. and Fagg, G.E., Differing effects of α-difluoromethylornithine and CGP 40116 on polyamine levels and infarct volume in a rat model focal cerebral ischaemia. Neurosci. Lett., 141 (1992) 131-135.

27. Seiler, N., Pharmacological properties of the natural polyamines and their depletion by biosynthesis inhibitors as a therapeutic approach. Prog. Drug Res., 37 (1991) 107-159.

28. Seiler, N., Polyamine oxidase, properties, and functions. Prog. Brain Res., 106 (1995) 333-344.

29. Tabor, C.W. and Tabor, H., Polyamines. Annu. Rev.Biochem., 53 (1984) 749-790.

30. Tadolini, B., Polyamine inhibition of lipoperoxidation: the influence of polyamines on iron oxidation in the presence of compounds mimicking phospholipid heads. Biochem. J.,249 (1998) 33-36.

31. Tamura, A., Graham, D.I., McCulloch, J. and Teasdale, G.M., Focal cerebral ischaemia in the rat: I. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J. Cereb. Blood Flow Metab., 1 (1981) 53-60.