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Effects of MDL 72527, a Specific Inhibitor of Polyamine Oxidase, on Brain Edema, Ischemic Injury Volume, and Tissue Polyamine Levels in Rats After Temporary Middle Cerebral Artery Occlusion

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Abstract: The possible effects of the polyamine interconversion pathway on tissue polyamine levels, brain edema formation, and ischemic injury volume were studied by using a selective irreversible inhibitor, MDL 72527, of the interconversion pathway enzyme, polyamine oxidase. In an intraluminal suture occlusion model of middle cerebral artery in spontaneously hypertensive rats, 100 mg/kg MDL 72527 changed the brain edema formation from 85.7 ± 0.3 to 84.5 ± 0.9% in cortex ($p < 0.05$) and from 79.9 ± 1.7 to 78.4 ± 2.0% in subcortex (difference not significant). Ischemic injury volume was reduced by 22% in the cortex ($p < 0.05$) and 17% in the subcortex ($p < 0.05$) after inhibition of polyamine oxidase by MDL 72527. There was an increase in tissue putrescine levels together with a decrease in spermine and spermidine levels at the ischemic site compared with the nonischemic site after ischemia–reperfusion injury. The increase in putrescine levels at the ischemic cortical and subcortical region was reduced by a mean of 45% with MDL 72527 treatment. These results suggest that the polyamine interconversion pathway has an important role in the postischemic increase in putrescine levels and that blocking of this pathway can be neuroprotective against neuronal cell damage after temporary focal cerebral ischemia. **Key Words:** Cerebral edema—Cerebral ischemia—Polyamine interconversion pathway—MDL 72527—Polyamine—Polyamine oxidase.
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Putrescine, spermidine, and spermine, which are endogenous polyamines, are essential for many cellular growth processes, such as proliferation, regeneration, and differentiation (Pegg and McCann, 1982). Polyamine levels are modulated by changes in ornithine decarboxylase (ODC) activity. This is the primary rate-limiting step in the generation of polyamines, which acts by catalyzing the metabolism of ornithine to putrescine (Dienel et al., 1985). Numerous stresses and stimuli such as seizures, excitotoxic conditions, and traumatic brain injury increase the activity of the ODC enzyme (Pajunen et al., 1979; Gardiner and de Belleroche, 1990; Martinez et al., 1991; Baskaya et al., 1996). It has also been shown that reversible cerebral ischemia resulted in a delayed postischemic increase in ODC activity that is accompanied by a postischemic overshoot in brain putrescine levels (Paschen et al., 1987*a*, 1991; Dempsey et al., 1988). It has been suggested that the increase in putrescine content is the result of the changes in the activity of two key enzymes in polyamine metabolism, namely, an increase in production through ODC and a decrease in metabolism through *S*-adenosylmethionine decarboxylase activity (Kleihues et al., 1975; Dienel et al., 1985). These changes result in an overshoot in the formation of putrescine, whereas the conversion of putrescine to spermidine and spermine is inhibited.

Recent findings, however, suggest that the postischemic overshoot in putrescine formation is only partly caused by the increase in ODC activity (Paschen et al., 1988*b*, 1990, 1993). It is well known that cellular polyamine levels are also controlled by regulation of the rate of polyamine acetylation by spermine/spermidine N^1-acetyltransferase followed by oxidation via polyamine oxidase (PAO). These reactions result in the interconversion of spermine back into putrescine. This has led us to speculate that the interconversion pathway also plays a role in the resultant increase in putrescine levels after

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Abbreviations used: CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery; MDL 72527, N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine · 2HCl; ODC, ornithine decarboxylase; PAO, polyamine oxidase; rCBF, regional cerebral blood flow; SHR, spontaneously hypertensive rat.

cerebral ischemia. This may also contribute to ischemia-related neuronal death because such polyamine oxidation results in toxic by-products such as hydrogen peroxide (Seiler, 1995), which has been implicated in cell damage.

The purpose of the present study is to test the hypothesis that MDL 72527 [N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine · 2HCl], a specific inhibitor of PAO, reduces putrescine and ischemic injury in the rat brain following transient ischemia. In this study, spontaneously hypertensive rats (SHRs) were used because we have previously shown that intraluminal middle cerebral artery (MCA) occlusion in SHRs is associated with a more consistent, reliable, and reproducible volume of ischemic injury (Dogan et al., 1998).

MATERIALS AND METHODS

In this study, we carefully adhered to the animal welfare guidelines set forth in the *Guide for the Care and Use of Laboratory Animals* (U.S. Department of Health and Human Services publication 85-23, 1985). The animals were operated on in random order, and outcome assessments were made by investigators blinded to the experimental group.

Materials

MDL 72527, a specific PAO inhibitor, was obtained from Hoechst Marion Roussel (Bridgewater, NJ, U.S.A.). All the other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Experimental protocol

All rats (n = 59) underwent 2 h of ischemia with 22 h of reperfusion. To measure brain polyamine levels, cerebral edema formation, and ischemic injury volume, animals were killed 24 h after the onset of the ischemic event.

MDL 72527 or physiological saline was administered intraperitoneally 5 min after the induction of ischemia. Although it has been shown that 20 mg/kg MDL 72527 was enough to block PAO completely in the normal brain (Seiler and Bolkenius, 1985), in the present study, 100 mg/kg MDL 72527 was used because of the possible further activation of the polyamine interconversion pathway after transient cerebral ischemia. All animals received the same amount of fluid (0.5 ml).

Preparation of animals

Male SHRs weighing 250–300 g were anesthetized with 2% halothane for induction and 1.2% halothane plus 50% nitrous oxide/50% oxygen for maintenance. Animals were ventilated mechanically with a rodent ventilator (rodent ventilator model 683; Harvard, South Natick, MA, U.S.A.) through an endotracheal tube (PE-240 polyethylene tubing). P_aO_2 was maintained between 100 and 200 mm Hg, and P_aCO_2 was kept between 30 and 40 mm Hg. The left femoral artery was cannulated for continuous arterial blood pressure monitoring and to obtain measurements of pH, P_aCO_2, P_aO_2, hemoglobin, and blood glucose concentration (i-STAT; Sensor Devices, Waukesha, WI, U.S.A.). Temporalis muscle and rectal temperature probes were inserted, and the cranial and body temperatures were maintained in a physiological range with a heating blanket and lamp.

Regional cerebral blood flow (rCBF) measurement

Changes in rCBF were recorded at the surface of the left parietal cortex using laser Doppler flowmeter probes attached to a laser flowmeter device (Laserflo blood perfusion monitor BPM 403A; TSI Inc., St. Paul, MN, U.S.A.). After the rats were placed in a stereotaxic frame, a craniectomy (6 mm in diameter, 2–4 mm lateral and 1–2 mm caudal to the bregma) was performed with extreme care over the MCA territory using a trephine. The dura was left intact. The probe of the laser Doppler flowmeter (model PD-434; Vasamedics, L.L.C., St. Paul) was placed on the cortical surface and fixed to the periosteum with 4-0 silk suture. Changes in rCBF were expressed as a percentage from baseline rCBF.

Focal ischemia model

MCA occlusion was induced as described by Longa et al. (1989). In brief, the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were isolated through a ventral midline incision. A 3-0 nylon monofilament, with its tip rounded by heating near a flame, was introduced into the ECA lumen through a puncture at the ECA and then gently advanced from the ECA to the ICA until reduction of rCBF was seen. Two hours after MCA occlusion, the suture was withdrawn back into the ECA to restore CCA–ICA–MCA blood flow. The incision was closed after 90 min of recirculation. The animals were then permitted to recover from the anesthesia at room temperature.

Measurement of tissue polyamine levels

After the animals were killed, their brains were quickly removed, and right and left cerebral cortical and striatal tissues were dissected. These samples were immediately frozen in liquid N_2 and stored at −70°C until assay. Frozen brain tissue was homogenized in 0.2 *M* perchloric acid and then centrifuged at 16,000 *g* for 20 min. Polyamines in the supernatant were quantified as described earlier (Kabra et al., 1986) with minor modifications. The supernatants were dansylated and applied to Bond-Elut C-18 columns (Varian Associates, Harbor City, CA, U.S.A.). Samples were injected into a Hewlett-Packard model 1100 HPLC apparatus. The polyamines were quantified against internal (1,7-diaminoheptane) and external standards using a Hewlett-Packard model 1046A fluorescence detector with λ_{ex} 340 nm and λ_{em} 515 nm. Polyamine levels were expressed as picomoles per gram of tissue based on wet weight.

Wet–dry method for cerebral edema measurements

After the animals were killed, their brains were removed, and left and right cerebral cortical and striatal tissues were dissected and weighed immediately to yield wet weight. After drying in a desiccating oven at 70°C for 48 h, they were reweighed for the determination of water content. Water content is expressed as percent H_2O, which is calculated as [(wet weight − dry weight)/wet weight] × 100.

Measurement of volume of ischemic brain injury

After the animals were decapitated, their brains were quickly removed, placed in cold saline solution for 10 min, and then cut into 7- × 2-mm coronal slices in a rodent brain matrix (RBM 4000C; Activational Systems, Warren, MI, U.S.A.). Sections were stained with 2% 2,3,5-triphenyltetrazolium chloride monohydrate as previously described (Bederson et al., 1986). The volume of ischemic brain injury was measured using a Macintosh computer using the public domain NIH Image program (written by Wayne Rasband at the U.S. National Institutes of Health and available from the Internet by anonymous ftp from zippy.nimh.nih.gov or a floppy disk from NTIS, 5285 Port Royal Road, Springfield, VA 22161, U.S.A., part no. PB93-504868). The volume of ischemic brain injury was calculated by the numeric integration of data from individual slices. To

TABLE 1. *Physiological parameters in rats treated with saline or MDL 72527*

	pH	P_{CO_2} (mm Hg)	P_{O_2} (mm Hg)	Hb (g/dl)	Gl (mg/dl)	Temperature (°C)		Mean arterial blood pressure (mm Hg)		
						Muscle	Rectal	Preischemia	Ischemia	Reperfusion
Saline	7.43 ± 0.04	34.3 ± 3.4	133 ± 24	13 ± 1.1	91 ± 5	36.7 ± 0.4	36.9 ± 0.4	130 ± 17	125 ± 17	121 ± 14
MDL 72527	7.42 ± 0.03	34.8 ± 3.9	145 ± 30	13 ± 1.2	88 ± 8	36.7 ± 0.2	37.4 ± 0.3	121 ± 6	116 ± 18	113 ± 11

Data are mean ± SD values. There was no significant difference between the groups in any physiological parameter. Hb, hemoglobin; Gl, glucose.

compensate for brain swelling in the ischemic hemisphere (Swanson et al., 1990), we corrected infarct volume in each rat by computing the volume of the left and right hemispheres and applying the following formula: corrected infarct volume = right hemisphere volume − (left hemisphere volume − measured infarct volume).

Statistical analysis

Data are expressed as mean ± SD values and were analyzed by a repeated-measures ANOVA followed by contrasts in repeated-measures design and Student's *t* test (Super ANOVA; Abacus Concepts, Berkeley, CA, U.S.A.). A p value of <0.05 was considered statistically significant.

RESULTS

Physiological parameters

There were no statistically significant differences regarding pH, P_aCO_2, P_aO_2, hemoglobin level, blood glucose concentration, or temporalis muscle or rectal muscle temperatures during the procedure (Table 1). Although MDL 72527 treatment significantly reduced mean arterial blood pressure compared with the saline treatment at 30 min, there was no significant difference between the two groups during the whole procedure according to the repeated-measures ANOVA.

Effect of MDL 72527 on rCBF

Occlusion reduced cortical blood flow to 20 ± 11% of baseline in the ischemic hemisphere of the saline-treated rats (n = 11) immediately at the time of ischemia. In the animals treated with MDL 72527 (n = 10), rCBF fell to 13 ± 7% of baseline in the ischemic hemisphere at the same time after MCA occlusion (Fig. 1). The rCBF remained around 20% of baseline throughout the occlusion period, with no significant difference between the groups. Reperfusion restored rCBF to 101 ± 49 and 121 ± 32% of baseline levels in the saline- and MDL 72527-treated groups, respectively. There was no significant difference between the groups regarding the rCBF during reperfusion period.

Effect of MDL 72527 on brain polyamine levels

The putrescine concentration was 6.9 ± 2.8 and 17.1 ± 13.7 nmol/g at the nonischemic cortical and striatal regions, respectively (Fig. 2). Two hours of ischemia followed by 22 h of reperfusion resulted in a significant increase in putrescine levels to 25.7 ± 4.5 ($p < 0.0001$) and 35.6 ± 16 nmol/g ($p < 0.05$) at the ischemic cortical and striatal regions, respectively (n = 8). Treatment with MDL 72527 (n = 9) decreased the postischemic putrescine levels to 14.4 ± 2.8 nmol/g (44%; $p < 0.0001$ for saline vs. MDL) at the cortex and 19.5 ± 7.9 nmol/g (45%; $p < 0.05$ for saline vs. MDL) at the striatum. The effect of MDL 72527 on the tissue putrescine levels was found to be more pronounced at the nonischemic site. Putrescine levels were decreased by 75 and 65% in cortex and striatum, respectively, after MDL 72527 treatment.

There was a significant decrease in tissue spermidine and spermine levels at the ischemic cortex compared with the nonischemic cortex after ischemia–reperfusion injury (134.3 ± 19.5 and 241.2 ± 43 nmol/g, respectively, for spermidine, $p < 0.0001$; 90.6 ± 9.3 and 156.1 ± 26.2 nmol/g, respectively, for spermine, $p < 0.0001$). MDL 72527 did not significantly change the absolute value of spermidine and spermine after ischemia. However, PAO forms spermidine from spermine; therefore, the effect of MDL 72527 on spermidine levels was evaluated by the spermidine/spermine ratio, which was thought to be a more reliable measure of spermidine formation from spermine through the interconversion pathway (Seiler and Bolkenius, 1985). The ratio of spermidine/spermine was significantly lower after MDL 72527 treatment compared with the saline treatment (Fig. 2).

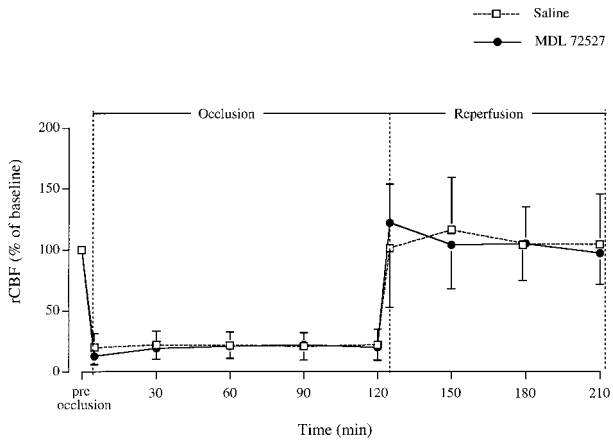

FIG. 1. Effects of intraperitoneal administration of saline (--□--) or MDL 72527 (—●—) on rCBF in the MCA occlusion model in SHRs. Changes in rCBF are expressed as a percentage of the baseline rCBF. Data are mean ± SD (bars) values. There was no significant difference between groups.

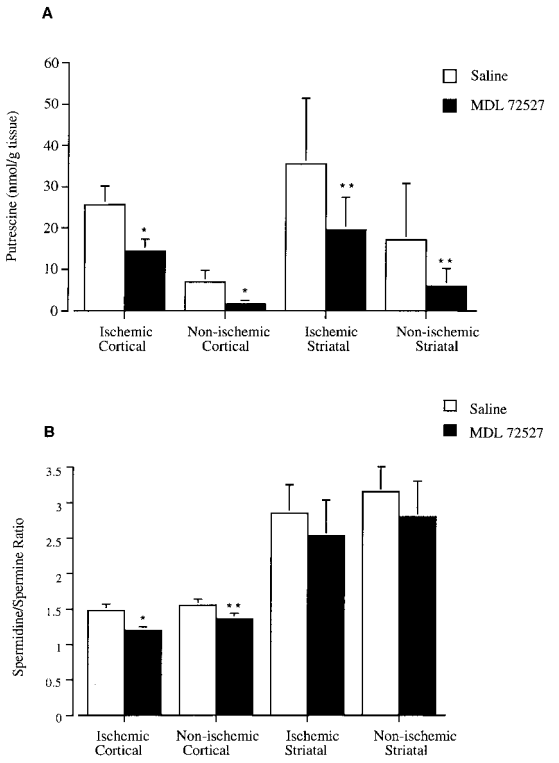

FIG. 2. Data are mean ± SD (bars) values. **A:** Effect of MDL 72527 on cortical and striatal putrescine levels at 24 h after ischemia–reperfusion injury. Treatment with MDL 72527 significantly decreased the putrescine levels in all regions. *$p < 0.0001$ versus saline-treated group; **$p < 0.05$ versus saline-treated group. **B:** Effect of MDL 72527 on spermidine/spermine ratio. *$p < 0.0001$, **$p < 0.0005$ versus saline treatment.

Effect of MDL 72527 on brain water content

Two hours of ischemia followed by 22 h of reperfusion resulted in a significant increase in brain water content of the ischemic cortex compared with the non-ischemic cortex (Fig. 3). However, in the ischemic hemisphere of the animals treated with MDL 72527 (n = 10), the cortical water content was significantly lower than in the animals treated with saline (n = 11; 84.5 ± 0.9 and 85.7 ± 0.3%, respectively; $p < 0.05$). Although not significant, there was a trend for striatal water content to be lower at the ischemic site of the animals treated with MDL 72527 than those of animals treated with saline (78.4 ± 2.0 vs. 79.9 ± 1.7%, respectively).

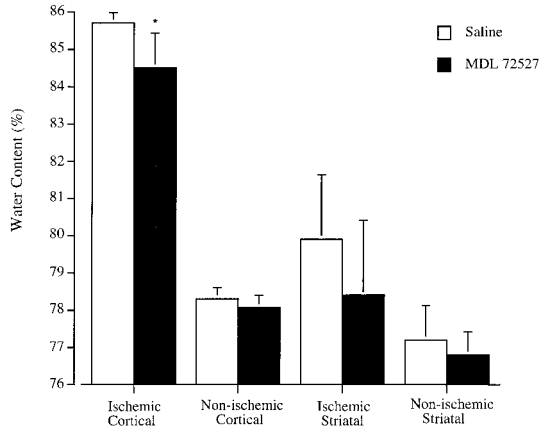

FIG. 3. Changes in water content of cortex and striatum. Treatment with MDL 72527 significantly decreased the water content of the ischemic cortex. Data are mean ± SD (bars) values. *$p < 0.05$ versus saline-treated group.

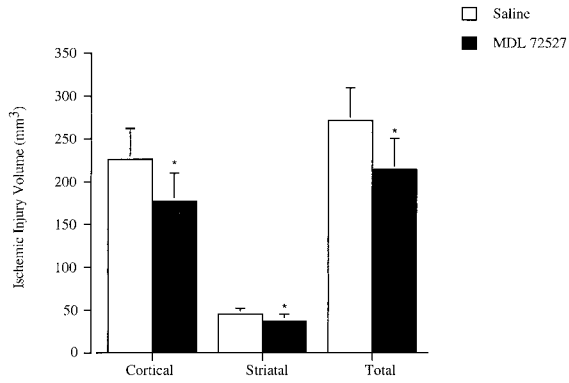

FIG. 4. Effect of MDL 72527 on volume of ischemic brain injury at 24 h after ischemia–reperfusion injury. Data are mean ± SD (bars) values. A statistically significant difference existed between the groups: *$p < 0.05$ versus saline-treated group.

Effect of MDL 72527 on ischemic brain injury volume

The total volume of ischemic brain injury was 271 ± 38 mm^3 in the saline-treated group (n = 11). Treatment with 100 mg/kg MDL 72527 (n = 10) reduced the ischemic injury volume to 214 ± 36 mm^3. In the cerebral cortex the volume of ischemic brain injury decreased by 22% and in the striatum 17% (Fig. 4). A statistically significant difference existed between the two groups for both regions (226 ± 36 to 177 ± 33 mm^3 for cortex, $p < 0.01$; 44 ± 7 to 37 ± 8 mm^3 for striatum, $p < 0.05$).

DISCUSSION

Polyamines, especially putrescine, have been implicated in blood–brain barrier breakdown and vasogenic brain edema induced by cold injury, global and focal cerebral ischemia, and traumatic brain injury. Tissue putrescine levels change to a greater degree than those of spermidine and spermine after these pathological conditions (Koenig et al., 1983*a*,*b*; Paschen et al., 1987*a*,b; Dempsey et al., 1988; Baskaya et al., 1996). Because the amount of ischemic cell damage correlates with the putrescine content in each region in the brain (Paschen et al., 1988*a*), it has been thought that putrescine is involved in these manifestations of ischemic cell damage (Paschen et al., 1987*a*). Because increased ODC activity always precedes the postischemic overshoot in putrescine concentrations, it is frequently taken as a measure of polyamine metabolism and thus as an indirect

indicator of putrescine production. There are, however, some further considerations in making this assumption:

(a) Paschen et al. (1990) have shown that there was a dissociation between the ODC activity and putrescine levels in the rat hippocampal CA1 subfield after barbiturate treatment. This is a brain region that shows extreme sensitivity to ischemia.

(b) It has been shown that after a 5-min duration of global ischemia, the increase in level of putrescine was most pronounced after 24 h of recirculation. This is after ODC activity had returned to control levels (Paschen et al., 1993).

(c) The specific ODC inhibitor difluoromethylornithine blocked the increase in ODC activity completely with only minor effects on increased putrescine levels after ischemia (Paschen et al., 1988*b*).

All of these findings suggest that the postischemic overshoot in putrescine content is only partly caused by the increase in ODC activity. A reduction in *S*-adenosylmethionine decarboxylase activity, the second enzyme of the polyamine pathway of metabolism, might be an important cause of the postischemic overshoot in putrescine levels after cerebral ischemia (Kleihues et al., 1975; Dienel et al., 1985). On the other hand, it is well known that cellular polyamine levels are also controlled by regulation of the rate of polyamine acetylation and oxidation. These processes are catalyzed by spermine/spermidine N^1-acetyltransferase and PAO, respectively, and result in the interconversion of spermidine back into putrescine with hydrogen peroxide and 3-acetaminopropanal as by-products (Seiler, 1995). Seiler and Bolkenius (1985) have studied the effect of 20 mg/kg MDL 72527 on polyamine levels and suggested that in the normal brain only ~30% of the putrescine is derived from ornithine by decarboxylation, whereas ~70% is formed by the interconversion of spermidine. In a similar manner, in the present study we found by a mean reduction of 75% in the putrescine levels in the nonischemic cortex by treatment with MDL 72527, a PAO inhibitor, compared with saline. As spermine/spermidine N^1-acetyltransferase is rapidly induced in the brain by several stimuli (Ientile et al., 1988; Gilad et al., 1992), the resultant increase in putrescine levels after cerebral ischemia also might be due to a temporary activation of the interconversion pathway.

Our study showed that there is a significant increase in tissue putrescine levels together with a decrease in spermine and spermidine levels in the ischemic cortex compared with the nonischemic cortex. The increase in putrescine levels at the ischemic cortical and subcortical region was reduced by a mean of 45% after MDL 72527 treatment. This treatment also slightly decreased the formation of spermidine from spermine, which was indicated by a lowered spermidine/spermine ratio. These findings suggest that there is an important activation at the interconversion pathway after transient focal cerebral ischemia and that this activation has an ~45% contribution to the delayed postischemic increase in tissue putrescine levels. It has been postulated for a long time that the increase in putrescine levels can contribute to the toxicity in ischemia. However, the recent studies showing the lack of neurotoxicity associated with overexpressing putrescine in transgenic mice (Lukkarainen et al., 1995) raise the question that increased activity of the polyamine interconversion pathway might be deleterious to neurons by the formation of hydrogen peroxide, a toxic by-product of polyamine oxidation (Seiler, 1995). Hydrogen peroxide is known to be toxic to many systems, including nervous tissue. It may lead to blood–brain barrier breakdown and cerebral edema (Tasdemiroglu et al., 1994; Kimelberg, 1995). Li et al. (1995) have demonstrated that 2 h of MCA occlusion followed by reperfusion resulted in DNA fragmentation, indicative of apoptosis along with ischemic tissue necrosis. The localization of the apoptotic cells is in the boundary zone of the infarction, suggesting that free radical formation on reoxygenation may have a role in inducing apoptosis after ischemia–reperfusion injury. Ha et al. (1997) have reported that free radical by-products of the polyamine interconversion pathway can be an important source to induce apoptosis. This mechanism is thought to be responsible for the antitumor activity of several polyamine analogues. They have demonstrated that MDL 72527 significantly reduces the amount of high-molecular-weight DNA fragmentation in N^1-ethyl-N^{11}-[(cyclopropyl)methyl]-4,8,-diazaundecane cytotoxicity in sensitive cells.

Postischemic apoptosis can develop as a relatively early event and contribute to the development of an ischemic infarct (Charriaut-Marlangue et al., 1995; Li et al., 1995). In the present study, we have shown that the blocking of the polyamine interconversion pathway by a PAO inhibitor significantly decreased the ischemic injury volume (22%) and brain water content, an indicator of brain edema, at the ischemic cortex after ischemia–reperfusion injury. Because MCA occlusion in SHRs usually results in larger infarct volume (Dogan et al., 1998) and SHRs are more refractory than normotensive rats to treatments aimed at reducing stroke size, the present findings indicate that the protection afforded by MDL 72527 is at least modest. We postulate that the protective effect of MDL 72527 may be due, at least in part, to inhibition of toxic by-products of polyamine interconversion pathway.

In conclusion, this report is the first demonstration that inhibition of PAO, the enzyme of the polyamine interconversion pathway, results in a lesser increase in putrescine content and reduced edema formation and ischemic injury volume after transient cerebral ischemia. These results suggest that the polyamine interconversion pathway may be activated after temporary focal cerebral ischemia and be as important as ODC activation in the postischemic overshoot in putrescine levels.

REFERENCES

Baskaya M. K., Rao A. M., Prasad M. R., and Dempsey R. J. (1996) Regional activity of ornithine decarboxylase and edema formation after traumatic brain injury. *Neurosurgery* **38,** 140–145.

Bederson J. B., Pitts L. H., Germano S. M., Nishimura M. C., Davis R. L., and Bartkowski H. M. (1986) Evaluation of 2,3,5-triphenyltetrazolium chloride as a stain for detection and quantification of experimental cerebral infarction in rats. *Stroke* **17,** 1304–1308.

Charriaut-Marlangue C., Margaill I., Plotkine M., and Ben-Ari Y. (1995) Early endonuclease activation following reversible focal ischemia in the rat brain. *J. Cereb. Blood Flow Metab.* **15,** 385–388.

Dempsey R. J., Combs D. J., Olson J. W., and Maley M. (1988) Brain ornithine decarboxylase activity following transient cerebral ischemia: relationship to cerebral edema development. *Neurol. Res.* **10,** 175–178.

Dienel G. A., Cruz N. F., and Rosenfeld S. J. (1985) Temporal profiles of proteins responsive to transient ischemia. *J. Neurochem.* **44,** 600–610.

Dogan A., Baskaya M. K., Rao V. L. R., Rao A. M., and Dempsey R. J. (1998) Intraluminal suture occlusion of the middle cerebral artery in spontaneously hypertensive rats. *Neurol. Res.* **20,** 265–270.

Gardiner I. M. and de Belleroche J. (1990) Reversal of neurotoxin-induced ornithine decarboxylase activity in rat cerebral cortex by nimodipine. A potential neuroprotective mechanism. *Stroke* **21** (Suppl. 12), 93–94.

Gilad G. M., Gilad V. H., Wyatt R. J., and Casero R. A. Jr. (1992) Chronic lithium treatment prevents the dexamethasone-induced increase of brain polyamine metabolizing enzymes. *Life Sci.* **50,** PL149–PL154.

Ha H. C., Woster P. M., Yager J. D., and Casero R. A. Jr. (1997) The role of the polyamine catabolism in polyamine analogue-induced programmed cell death. *Proc. Natl. Acad. Sci. USA* **94,** 11557–11562.

Ientile R., De Luca G., Di Giorgio R. M., and Macaione S. (1988) Glucocorticoid regulation of spermidine acetylation in the rat brain. *J. Neurochem.* **51,** 677–682.

Kabra P. M., Lee H. K., Lubich W. P., and Marton L. J. (1986) Solid phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phase liquid chromatography: improved separation systems for polyamines in cerebrospinal fluid, urine and tissue. *J. Chromatogr.* **380,** 19–32.

Kimelberg H. K. (1995) Current concepts of brain edema. Review of laboratory investigations. *J. Neurosurg.* **83,** 1051–1059.

Kleihues P., Hossmann K.-A., Pegg A. E., Kobayashi K., and Zimmerman V. (1975) Resuscitation of the monkey brain after one hour complete ischemia; indications of metabolic recovery. *Brain Res.* **95,** 61–73.

Koenig H., Goldstone A., and Lu C. Y. (1983*a*) Polyamines regulate calcium fluxes in a rapid plasma membrane response. *Nature* **305,** 530–534.

Koenig H., Goldstone A. D., and Lu C. Y. (1983*b*) Blood–brain barrier breakdown in brain edema following cold injury is mediated by microvascular polyamines. *Biochem. Biophys. Res. Commun.* **116,** 1039–1045.

Li Y., Chopp M., Jiang N., Yao F., and Zaloga C. (1995) Temporal profile of in situ DNA fragmentation after transient middle cerebral artery occlusion in the rat. *J. Cereb. Blood Flow Metab.* **15,** 389–397.

Longa E. Z., Weinstein P. R., Carlson S., and Cummins R. (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* **20,** 84–91.

Lukkarainen J., Kauppinen R. A., Koistinaho J., Halmekyto, Alhonen L. M., and Janne J. (1995) Cerebral energy metabolism and immediate early gene induction following severe incomplete ischaemia in transgenic mice overexpressing the human ornithine decarboxylase gene: evidence that putrescine is not neurotoxic in vivo. *Eur. J. Neurosci.* **7,** 1840–1849.

Martinez E., de Vera N., and Artigas F. (1991) Differential response of rat brain polyamines to convulsant agents. *Life Sci.* **48,** 77–84.

Pajunen A. E., Hietala O. A., Baruch-Virransalo E. L., and Piha R. S. (1979) The effect of DL-allylglycine on polyamine and GABA metabolism in mouse brain. *J. Neurochem.* **32,** 1401–1408.

Paschen W., Schmidt-Kastner R., Djuricic B., Meese C., Linn F., and Hossmann K.-A. (1987*a*) Polyamine changes in reversible cerebral ischemia. *J. Neurochem.* **49,** 35–37.

Paschen W., Hallmayer J., and Mies G. (1987*b*) Regional profile of polyamines in reversible cerebral ischemia of Mongolian gerbils. *Neurochem. Pathol.* **7,** 143–156.

Paschen W., Hallmayer J., and Rohn G. (1988*a*) Relationship between putrescine content and density of ischemic cell damage in the brain of Mongolian gerbils: effect of nimodipine and barbiturate. *Acta Neuropathol. (Berl.)* **76,** 388–394.

Paschen W., Rohn G., Meese C. O., Djuricic B., and Schmidt-Kastner R. (1988*b*) Polyamine metabolism in reversible cerebral ischemia: effect of alpha-difluoromethylornithine. *Brain Res.* **453,** 9–16.

Paschen W., Hallmayer J., Mies G., and Rohn G. (1990) Ornithine decarboxylase activity and putrescine levels in reversible cerebral ischemia of Mongolian gerbils: effect of barbiturate. *J. Cereb. Blood Flow Metab.* **10,** 236–242.

Paschen W., Csiba L., Rohn G., and Bereczki D. (1991) Polyamine metabolism in transient focal ischemia of rat brain. *Brain Res.* **566,** 354–357.

Paschen W., Cleef M., Rohn G., Muller M., and Pajunen A. E. (1993) Ischemia-induced disturbances of polyamine synthesis. *Prog. Brain Res.* **96,** 147–160.

Pegg A. E. and McCann P. P. (1982) Polyamine metabolism and function. *Am. J. Physiol.* **243,** C212–C221.

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

Seiler N. and Bolkenius F. N. (1985) Polyamine reutilization and turnover in brain. *Neurochem. Res.* **10,** 529–544.

Swanson R. A., Morton M. T., Tsao-Wu G., Savalos R. A., Davidson C., and Sharp F. R. (1990) A semiautomated method for measuring brain infarct volume. *J. Cereb. Blood Flow Metab.* **10,** 290–293.

Tasdemiroglu E., Christenberry P. D., Ardell J. L., Chronister R. B., and Taylor A. E. (1994) Effects of antioxidants on the blood brain barrier and postischemic hyperemia. *Acta Neurochir. (Wien)* **131,** 302–309.