Original Article

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Effects of intravenous dimethyl sulfoxide on ischemia evolution in a rat permanent occlusion model

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Abstract

Dimethyl sulfoxide (DMSO) has a variety of biological actions that suggest efficacy as a neuroprotectant. We (1) tested the neuroprotective potential of DMSO at different time windows on infarct size using 2,3,5-triphenyltetrazolium staining and (2) investigated the effects of DMSO on ischemia evolution using quantitative diffusion and perfusion imaging in a permanent middle cerebral artery occlusion (MCAO) model in rats. In experiment 1, DMSO treatment (1.5 g/kg intravenously over 3 h) reduced infarct volume 24 h after MCAO by 65% ($P<0.00001$) when initiated 20 h before MCAO, by 44% ($P=0.0006$) when initiated 1 h after MCAO, and by 17% ($P=0.11$) when started 2 h after MCAO. Significant infarct reduction was also observed after a 3-day survival in animals treated 1 h after MCAO ($P=0.005$). In experiment 2, treatment was initiated 1 h after MCAO and maps for cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) were acquired before treatment and then every 30 mins up to 4 h. Cerebral blood flow characteristics and CBF-derived lesion volumes did not differ between treated and untreated animals, whereas the ADC-derived lesion volume essentially stopped progressing during DMSO treatment, resulting in a persistent diffusion/perfusion

mismatch. This effect was mainly observed in the cortex. Our data suggest that DMSO represents an interesting candidate for acute stroke treatment.

Keywords:

diffusion/perfusion mismatch, dimethyl sulfoxide, focal brain ischemia, neuroprotection

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Introduction

Dimethyl sulfoxide (DMSO) is widely used as a solvent for a variety of drugs and has a broad spectrum of biological activities that suggest efficacy as a neuroprotectant. These activities include hydroxyl radical scavenging (Repine *et al*, 1981), anti-inflammatory (Wood and Wood, 1975) and anti-edema effects (Camp *et al*, 1981), impairment of platelet aggregation and adhesiveness (Dujovny *et al*, 1983; Schiffer *et al*, 1976), and prevention of glutamate-induced neuronal cell death (Lu and Mattson, 2001).

However, prior reports on the effect of DMSO on focal cerebral ischemia were contradictory. Some investigators reported substantial improvements in mortality, morbidity, and infarct volume (de la Torre and Surgeon, 1976; Laha *et al*, 1978; Shimizu *et al*, 1997), while others failed to confirm these findings (McGraw, 1977; Little *et al*, 1981). Thus, the beneficial effects of DMSO in the treatment of acute focal brain ischemia are not clearly delineated.

In circumstances where neuroprotective effects of DMSO have been reported, the mechanisms for the salubrious effects are poorly understood. Most of the prior studies on the neuroprotective effects of DMSO were snapshot measurements performed using classical histologic methods (de la Torre and Surgeon, 1976; McGraw, 1977; Laha *et al*, 1978; Shimizu *et al*, 1997). None of these studies investigated the dynamic *in vivo* effects of DMSO on the evolution of ischemic tissue injury; and none of these studies examined different time points of drug administration relative to ischemia onset in the same experimental setting. Furthermore, it remains unclear whether DMSO increases cerebral blood flow (CBF) to prevent injury in focal brain ischemia. Indeed, two studies claimed opposite effects of DMSO on CBF (Little *et al*, 1981; de la Torre, 1991).

Diffusion- (DWI) and perfusion-weighted (PWI) magnetic resonance imaging are powerful imaging modalities for early detection of cerebral ischemia (Hoehn-Berlage *et al*, 1995). The region of the ischemic brain with diminished perfusion but without altered diffusion characteristics (diffusion/perfusion mismatch) is thought to represent an approximation of the ischemic penumbra. Agents that favor the survival of the penumbra represent an attractive therapeutic approach to acute stroke (Schlaug *et al*, 1999).

The purpose of the present study was to investigate systematically the effects of intravenous DMSO in an established model of permanent ischemia in rats. Specifically, we (1) evaluated the neuroprotective potential of DMSO at different times relative to the onset of focal cerebral ischemia; (2) studied the effect of DMSO on the spatio-temporal evolution of the ischemic lesion using quantitative perfusion and diffusion imaging; and (3) investigated the influence of DMSO on CBF characteristics.

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Methods

Animal Preparations

All procedures used in this study were performed in accordance with our institutional guidelines and all experiments were performed in a masked, randomized manner. Male Wistar-Kyoto rats (n=79, 300 to 350 g, Taconic Farms, Germantown, NY, USA) were used for all experiments, after an overnight fast. Anesthesia was induced with an intraperitoneal injection of chloral hydrate (400 mg/kg). PE-50 tubing was inserted into the right femoral vein for drug infusions. Another PE-50 tubing was inserted into the right femoral artery for monitoring of systemic arterial blood pressure and heart rate (Biopac, Santa Barbara, CA, USA), and for obtaining blood samples to measure pO_2, pCO_2, pH, and plasma glucose (I-STAT, East Windsor, NJ, USA). Blood sampling was performed before and 3 h after occlusion. Rectal temperature was monitored and kept at 37°C±0.5°C throughout the entire study using a feedback-controlled heating pad. Although brain temperature has been shown to be slightly different from core temperature, rectal temperature has been proposed to reflect the changes in brain temperature (Maier *et al*, 1998). Furthermore, the stable apparent diffusion coefficient (ADC) values in the normal left hemisphere in both groups (see results) indicated that the brain temperature was stable and did not differ between groups (Hasegawa *et al*, 1994).

Permanent focal cerebral ischemia was produced by intraluminal suture occlusion of the right middle cerebral artery (MCAO). Briefly, a 4-0 silicone-coated nylon filament with a thermically rounded tip was introduced into the distal common carotid artery, which was then advanced via the internal carotid artery until mild resistance indicated that the tip was lodged in the anterior cerebral artery, and thus blocked blood flow to the MCA.

Study Design

The study consisted of two different experiments:

Experiment 1 aimed to investigate the neuroprotective potential of DMSO when initiated at different times relative to the onset of ischemia. Infarct volume was defined at 24 h after MCAO using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO, USA) was diluted to a 33% solution with saline. All treatment animals received a 3-h intravenous infusion at a total dose of 1.5 g/kg DMSO (total volume ~1.5 mL). Control animals were administered a 3-h infusion of 1.5 mL saline. Animals were randomized to one of the following groups: start of infusion 20 h before induction of MCAO (groups DMSO-20 h (n=8) and saline-20 h (n=6)), start of infusion 1 h after MCAO (groups DMSO+1 h (n=8) and saline+1 h (n=8)), and start of infusion 2 h after MCAO (groups DMSO+2 h (n=8) and saline+2 h (n=6)).

To examine the long-term effects of DMSO, TTC-staining was performed after a 3-day survival period in two additional groups treated 1 h after ischemia (1.5 g/kg DMSO, n=6; saline, n=6).

Furthermore, the effects of DMSO on infarct size at 24 h after occlusion were evaluated when DMSO was administered at half dose (0.75 g/kg) starting 1 h after occlusion (n=8).

In *experiment 2*, the *in vivo* effects of DMSO on ADC and CBF characteristics and the spatio-temporal evolution of the ischemic lesion were evaluated. Immediately after occlusion, the animals were placed into the magnet, and anesthesia was switched to 1% isoflurane delivered in air at 1.5 L/min. Arterial blood pressure, heart rate, and rectal temperature were continuously monitored and temperature was maintained at 37.0°C±0.5°C using a feedback-controlled heating pad. Dimethyl sulfoxide (1.5 g/kg) was administrated intravenously over 3 h starting 1 h after MCAO (n=7, identical protocol to DMSO+1 h group in experiment 1). Control animals (n=8) received 1.5 mL saline. Magnetic resonance imaging data were acquired initially at 45 mins (baseline), at 90 mins after MCAO, and then every 30 mins up to 4 h after occlusion. 2,3,5,-Triphenyltetrazolium chloride-staining was performed 24 h after MCAO.

Magnetic Resonance Imaging Measurements

Magnetic resonance imaging was performed on a Bruker 4.7T/40 cm horizontal magnet (Billerica, MA, USA), and a 20 G/cm magnetic field gradient insert. A surface coil was used for brain imaging and an actively decoupled neck coil was used for CBF labeling. Coil-to-coil electromagnetic interaction was actively decoupled.

The average apparent diffusion coefficient (ADC_{av}) of water was obtained by averaging three ADC maps acquired separately with diffusion-sensitive gradients applied along the x, y, or z direction. Single-shot, spin-echo, and echo-planar images (EPI) were acquired with a 64 ×64 matrix, 20.3 ×20.3 mm^2 field of view (FOV), eight 1.5-mm slices, TR=2 secs, b=10 and 1270 secs/mm^2, TE=39 ms, Δ=17.5 ms, δ=5.6 ms, and 16 averages (total acquisition time 2.5 mins).

Noninvasive quantitative CBF measurements were performed using the continuous arterial spin-labeling (ASL) technique (Duong *et al*, 2000). Paired images were acquired alternately, one with arterial spin-labeling and the other without spin-labeling preparation (control). Single-shot, gradient-echo EPI were acquired with a 64 ×64 matrix, 20.3 ×20.3 mm^2 FOV, eight 1.5-mm slices, TR=2 secs, TE=15 ms. The continuous arterial spin used a 1.7-sec square RF pulse, in the presence of a 1.0 G/cm gradient, applied to the neck labeling coil on alternate acquisitions. A total of 120 pairs of ASL scans were acquired in two separate sets of 60 pairs each, one set obtained before, and the other after the ADC measurements (total acquisition time 8.9 mins).

To provide anatomical localization, we acquired T2-weighted images using the fast spin-echo pulse sequence with a 128 ×128 matrix, 20.3 ×20.3 mm^2 FOV, eight 1.5-mm slices, TR=2 secs, effective TE=80 ms, echo train length 16, and 16 signal averages.

Neurobehavior and Postmortem Evaluation

Twenty-four hours after MCAO, the animals were scored neurologically according to the following 6-point scale modified from the original description by Menzies *et al* (1992):

0=no apparent deficit, 1=failure to extend left forepaw fully, 2=decreased grip of the left forelimb while tail pulled, 3=spontaneous movement in all directions, circling to the left only if pulled by tail, 4=spontaneous circling to the left or no spontaneous walking with reduced level of consciousness, 5=dead.

Animals were allowed to survive 24 h after ischemia (except in the 3-day survival experiment). Brains were quickly removed and cut into eight 1.5-mm-thick coronal slices starting 1 mm from the frontal pole. Histologic staining was performed using TTC. The stained sections were then photographed and infarct volumes were determined using ImageJ software (Rasband, http://rsb.info.nih.gov/ij/). To compensate for the effects of brain edema, a corrected infarct volume was calculated, as previously described (Meng *et al*, 2004).

Data Analysis

MRI measurements were analyzed using the imaging processing programs Matlab (math-Works, Natick, MA, USA) and STIMULATE (Strupp, 1996). Quantitative ADC_{av} and CBF maps were calculated, as previously described in detail (Shen *et al*, 2003).

Calculation of *In Vitro* Lesion Size

Apparent diffusion coefficient and CBF viability thresholds, previously established and validated in our lab (Shen *et al*, 2003; Meng *et al*, 2004), were used to identify all pixels with abnormal ADC or CBF characteristics on each of the eight imaged slices at each time point. The corresponding ADC and CBF lesion volumes were then calculated by summing the abnormal area and multiplying by the slice thickness. The viability thresholds were 0.53×10^{-3} mm^2/sec for ADC and 0.30 mL/g min for CBF (Meng *et al*, 2004).

To investigate whether the DMSO effects on ischemic lesion evolution may be different between cortical and subcortical regions, the lesion progression in these two regions was also analyzed separately. The two regions of interest, the cortex and the striatum with basal forebrain, were drawn on anatomical scans with reference to a rat brain atlas.

Quantitative Apparent Diffusion Coefficient and Cerebral Blood Flow Values

The time course of quantitative ADC and CBF values of the ischemic and the normal hemisphere were analyzed. The effects of DMSO on the temporal evolution of the CBF and ADC characteristics of the initial core and mismatch regions, defined at 45 mins before treatment, were also evaluated. On the initial ADC and CBF maps (45 mins), ischemic damage was classified into the core area (ADC and CBF<thresholds) and the mismatch area (ADC>threshold, CBF<threshold). Subsequent ADC and CBF values were then prospectively measured in each area at each time point.

Statistical Analysis

Data are presented as mean±standard deviation. Statistical analysis was performed with repeated-measures ANOVA for continuous variables. A two-tailed unpaired *t*-test was

used to compare the parametric values. A two-tailed value of $P<0.05$ was considered significant.

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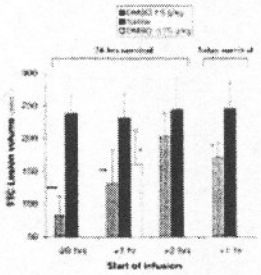
Results

Experiment 1

Physiologic variables are shown in Table 1 . All data were within the normal range and did not vary significantly among different groups or at different time points ($P>0.05$). The corrected TTC-defined infarct volumes 24 h after MCAO for each group are illustrated in Figure 1. DMSO (1.5 g/kg) resulted in a highly significant reduction in infarct size when the infusion was initiated 20 h before (65% reduction; $P<0.00001$) or 1 h after (44% reduction; $P=0.0006$) occlusion. The infarct volume was also smaller in rats treated at 2 h after MCAO, but this was not significant ($P=0.11$).

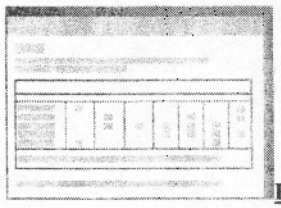
Figure 1.



2,3,5-Triphenyltetrazolium chloride (TTC) -defined corrected infarct volume 24 or 72 h after permanent middle cerebral artery occlusion (MCAO). Data are expressed as mean±s.d. $^{*}P<0.01$; $^{**}P<0.001$; $^{***}P<0.00001$.

Full figure and legend (49K)

Table 1 - Physiologic data before occlusion (baseline) and 3 or 4 h after MCAO.



Full table

Dimethyl sulfoxide at a dose of 0.75 g/kg initiated 1 h after occlusion also resulted in a significant infarct reduction (31% reduction; $P=0.008$), but the extent of neuroprotection was less robust compared with the higher dose of 1.5 g/kg ($P=0.24$).

In the 3-day survival group (1.5 g/kg DMSO starting 1 h after MCAO), 50% of the untreated animals died prematurely between 48 and 72 h after MCAO, whereas no rat died prematurely in the DMSO group. 2,3,5-Triphenyltetrazolium chloride staining in rats that died prematurely was performed within 3 h after death. Infarct volume was

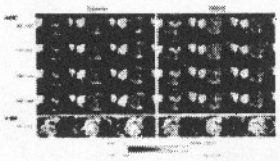
significantly reduced in the DMSO-treated animals (30% reduction, P=0.005, Figure 1). There was a trend towards an increase in infarct size after 3 days in treated animals compared with the 24-h survival DMSO group (P>0.05).

The neurologic scores 24 h after MCAO were significantly improved in the 20 h preocclusion treatment group (2.0±0.7 versus 3.5±0.6; P=0.0008), the 1 h postocclusion treatment group (2.3±0.7 versus 3.3±0.8; P=0.02), and the 1 h postocclusion treatment group that received half of the dose (2.5±0.5 versus 3.3±0.8; P=0.04). No significant difference in neurologic status was observed with the infusion starting 2 h after occlusion (2.7±0.8 versus 3.3±0.7; P=0.17).

Experiment 2

Mean arterial blood pressure, heart rate, and body temperature were comparable between the two groups and within the normal range throughout the study period (see also Table 1). Figure 2 shows the representative ADC and CBF maps from one control and one DMSO treated animal subjected to permanent MCAO.

Figure 2.



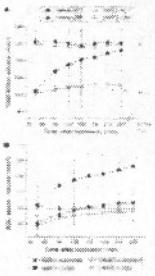
Representative apparent diffusion coefficient (ADC) and cerebral blood flow (CBF) maps from one control and one dimethyl sulfoxide (DMSO)-treated animal subjected to permanent middle cerebral artery occlusion (MCAO). Three of eight maps are shown at 45 mins (baseline), 120, 180, and 240 mins after occlusion for ADC maps, and at 45 mins for CBF maps. Dimethyl sulfoxide treatment was initiated 1 h after occlusion.

Full figure and legend (185K)

Temporal evolution of apparent diffusion coefficient- and cerebral blood flow-derived lesion volumes

Figure 3A shows the temporal evolution of lesion volumes over the 4-h imaging time period for both the treatment and the control groups. Cerebral blood flow-derived lesion volumes remained relatively constant in both groups without significant changes across time. No significant differences were observed between groups at any time point (P>0.05). In contrast, the temporal evolution of the ADC-derived lesion volumes differed significantly between the two groups. The initial ADC lesion volumes were essentially identical (129±41 versus 124±51 mm^3, P=0.8). While the ADC lesions continued to enlarge gradually over time in the untreated animals, lesion volumes in the DMSO-treated group increased only slightly at 90 mins and then largely stopped evolving. The ADC lesion at 4 h was 225±51 mm^3 in the control group, whereas it was 143±64 mm^3 in the DMSO group (P<0.05 for the group differences at 210 and 240 mins).

Figure 3.



(A) Temporal evolution of total apparent diffusion coefficient (ADC)- and cerebral blood flow (CBF)-derived lesion volumes (mean±s.d.) by using previously validated viability thresholds in permanently occluded rats. Dimethyl sulfoxide (DMSO) or saline infusion was initiated 1 h after occlusion. Saline group: CBF-lesion=closed diamonds, ADC-lesion=closed squares, 2,3,5-triphenyltetrazolium chloride (TTC)-lesion=gray square; DMSO group: CBF-lesion=open triangles, ADC-lesion=open circles, TTC-lesion=gray circle. **(B)** Temporal evolution of cortical and subcortical ADC lesion volumes (mean±s.d.). Saline lesion: cortex=closed circles, subcortex=closed squares; DMSO lesion: cortex=open circles, subcortex=open squares. $^{*}P<0.05$ for differences in ADC lesion volumes at same time points. $^{**}P=0.002$ for difference in TTC lesion volume at 24 h after occlusion.

Full figure and legend (46K)

As shown in Figure 3B, the treatment effect was maximal in the cerebral cortex as the cortical ADC-derived lesion volume completely stopped progressing during DMSO treatment. No significant differences in the evolution of the subcortical ADC lesions were observed.

The diffusion/perfusion mismatch region identified as the difference between the abnormal perfusion and diffusion regions was virtually identical between groups at baseline. During the infusion, the mismatch volume in the DMSO group was similar at all time points (ranging from 41% to 46% of the CBF deficit) and the abnormal perfusion volume was significantly larger than the abnormal diffusion volume at all time points ($P<0.02$ each). In the control group, the difference between the abnormal perfusion and diffusion regions was only significant at 45 and 90 mins after MCAO ($P<0.0001$ and $P<0.01$, respectively), and gradually decreased over time. By 4 h after occlusion, the abnormal diffusion volume was 94% of the abnormal perfusion volume.

Consistent with the results in experiment 1, the mean TTC-defined infarct volume 24 h after occlusion was significantly smaller in the DMSO-treated group ($P=0.002$, Figure 3A). Compared with the MRI lesion volumes at 4 h after MCAO, the TTC infarct volume in the control group was slightly larger than the ADC-derived lesion volume and essentially identical to the CBF-derived lesion. In the DMSO-treated animals, however, the TTC-defined infarct size 24 h after occlusion was virtually identical to the baseline ADC lesion before treatment (Figure 3A).

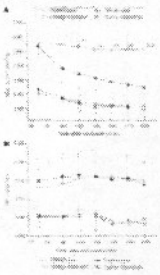
Quantitative apparent diffusion coefficient and cerebral blood flow values

In the nonischemic left hemisphere (LH), the mean CBF and ADC values remained constant in both groups without significant differences between groups or different time points ($P>0.05$). The average CBF and ADC were 1.07±0.19 mL/g min and 0.77±0.03 mm^2/sec in the untreated animals, and 1.04±0.16 mL/g min and 0.78±0.03 mm^2/sec in the DMSO-treated group, respectively.

In the ischemic right hemisphere (RH), the CBF was constantly reduced to an average of 36%±2% of the LH in the control animals ($P<0.0001$) and to an average of 37%±3% of the LH in the treated animals ($P<0.0001$). The ADC in the RH gradually decreased over time in the control group, whereas the decline in ADC was less pronounced in the DMSO group and stopped decreasing by 180 mins after MCAO.

The temporal evolution of ADC and CBF values in the core and mismatch regions defined before treatment were prospectively quantified (Figures 4A and 4B). At baseline, the ADC and CBF characteristics of the core and mismatch regions were comparable between the two groups ($P>0.05$). In the mismatch region, the mean ADC values in the control group continuously decreased over time. In the DMSO group, the ADC values in the mismatch area declined only slightly at 90 and 150 mins and then remained constant up to 4 h after occlusion. The difference in mean ADC values between the DMSO and the control group was significant at all time points during treatment ($P<0.05$ each). In the core region, no significant differences in the evolution of ADC values were observed between untreated and treated animals (Figure 4A).

Figure 4.



Temporal evolution of apparent diffusion coefficient (ADC) **(A)** and cerebral blood flow (CBF) **(B)** in the core and mismatch regions defined before treatment (45 mins) in permanently occluded rats. Dimethyl sulfoxide (DMSO) or saline was administered 1 h after occlusion. Dimethyl sulfoxide group: core=open triangle; mismatch=open circles. Saline group: core=closed triangles, mismatch=closed circles. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Full figure and legend (42K)

Region-specific CBF characteristics did not differ significantly between the two groups at any time point ($P>0.05$, Figure 4B). In both groups, a similar gradient of CBF reduction from the ischemic periphery towards the ischemic core was observed.

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Discussion

The present study demonstrated robust neuroprotection with DMSO in a widely used and established model of permanent focal cerebral ischemia. This effect was shown *in vivo* by diffusion and perfusion imaging, on neurologic scoring, and at postmortem by TTC staining at concentrations near those shown to be tolerated in humans.

Because immediate treatment is not clinically feasible in acute sporadic stroke, it is important to determine the therapeutic window. Dimethyl sulfoxide treatment initiated 1 h after occlusion resulted in a highly significant reduction of infarct size, whereas postischemic therapy after a 2-h delay showed only a trend towards neuroprotection. This agreed with the MRI findings, as the mismatch region in the untreated animals was significant up to 90 mins after MCAO but not at 2 h, implying that treatment initiated at 2 h is unlikely to show a significant reduction in infarct size. Moreover, the TTC infarct volume at 24 h in the non-MRI DMSO+2 h group was comparable with the ADC-derived lesion volume observed at 2 h in the MRI experiment control group, suggesting DMSO stopped further lesion growth and a rapid onset of action even with therapy initiated at 2 h. It is possible that higher concentrations of DMSO would provide a wider therapeutic window.

A long-lasting therapeutic effect was observed in the 3-day survival experiment when DMSO was administered 1 h after ischemia onset. Interestingly, the effect was less pronounced compared with the 24 h survival group. This suggests that part of the acute neuroprotection was only temporary. It is possible that repeated doses of intravenous DMSO would more effectively maintain neuroprotection. Nevertheless, DMSO therapy resulted in 100% survival for 3 days, whereas mortality rate in untreated animals was 50%. A 3-day period as an indicator for long-term effects was chosen because earlier studies demonstrated that there is no further increase in infarct volume 2 to 3 days after onset of ischemia in this stroke model (Rudin *et al*, 2001; van Lookeren Campagne *et al*, 1999). Furthermore, this permanent ischemia model is not ideal for examining longer survival periods because of the high mortality rate even in treated animals.

Previous studies on the effects of DMSO on focal brain ischemia have demonstrated contradictory results. In a permanent MCAO model in cats, de la Torre (1991) observed significant cell protection with 0.9 mg/kg intravenous DMSO administered 1 h after ischemia, whereas Little *et al* (1981) found no beneficial effects although treatment was initiated earlier (at occlusion) at an even higher dose (2.5 mg/kg intravenous). In rhesus monkeys, DMSO administered 4 h after MCAO reduced neurologic deficits and histopathologic changes (de la Torre and Surgeon, 1976), while treatment starting 30 mins after occlusion did not influence neurologic status or infarct size in baboons (Little *et al*, 1983). Interestingly, in dogs subjected to 6 h of embolic MCA occlusion, DMSO treatment (2 g/kg) beginning at the time of occlusion completely prevented cerebral infarction (Laha *et al*, 1978). The reasons for these discrepancies between different studies remain unclear, but differences in route of administration, timing, dosage, and stroke models used may be possible explanations. It will be important in future studies to use the paradigm outlined herein in a non-primate model of ischemia.

In the same permanent MCAO model as used in this study, Shimizu *et al* (1997) evaluated the effects of different DMSO doses when administered intraperitoneally 30 mins before occlusion. A significant reduction in infarct size, of approximately 36%, was achieved with doses ranging between 0.09 and 3 g/kg. Interestingly, this degree of neuroprotection is comparable with our findings in the animals where treatment was initiated 1 h after occlusion.

The initial pretreatment ADC lesion volume remained essentially constant in the DMSO-treated animals, while it progressively increased in the control group. This effect of 'ischemic lesion freezing' occurred mainly in the cortex. Because the CBF-derived lesion volumes remained unchanged, DMSO treatment resulted in a persistent diffusion/perfusion mismatch. Consistent with the persistent mismatch, the ADC in the mismatch zone remained relatively stable without a further decrease in DMSO-treated rats, whereas in the control group, the ADC in the mismatch area shifted towards the core zone values as ischemia progressed. In contrast, DMSO had no effect on the ADC characteristics in the core region, typically located in the striatum. These findings indicated that DMSO essentially exhibited its neuroprotective effects on tissue with still normal diffusion characteristics (mismatch tissue), and such tissues were primarily in the cortex, as confirmed by a complete cessation of lesion growth in the cortex.

In isolated dog brains subjected to complete ischemia, DMSO administration during reoxygenation resulted in a substantial increase in ATP associated with significantly lower lactate levels and incomplete metabolic recovery (Gilboe *et al*, 1991). Dimethyl sulfoxide has been reported to suppress neuronal synaptic activity and may consequently reduce cellular energy consumption (Nakahiro *et al*, 1992; Sawada and Sato, 1975). Dimethyl sulfoxide has also been shown to increase the latency of anoxic depolarization of hippocampal neurons after acute ischemia (Hulsmann *et al*, 1999). Peri-infarct depolarizations in the penumbral tissue, however, are accompanied by disruption of ion homeostasis and the need for metabolic energy to restore the ionic gradients, eventually leading to ATP depletion and irreversible cell damage (Back *et al*, 1995). These findings—an increase in ATP combined with reduced energy consumption—suggest that the protective effects of DMSO on tissue that has not yet advanced to bioenergetic failure (mismatch) may reflect favorable changes in the balance of energy supply and demand. These changes would maintain ion-pump function and membrane potential as well as preventing a further increase in lactate and tissue acidosis.

Two previous studies evaluated the effect of DMSO on the CBF in permanent MCAO in cats. Little *et al* (1981) found no effect of DMSO on CBF, accompanied with no effects on histopathology. However, de la Torre (1991) observed a sustained increase in CBF in the ischemic cortex by 27%, associated with cortical cell protection. Based on these findings, the authors concluded that the protective effects of DMSO may result, at least partially, from its ability to increase CBF in brain ischemia. Our data do not support this hypothesis. Using combined perfusion and diffusion imaging, we demonstrated that the neuroprotective effect of DMSO was not associated with an increase in CBF, neither hemispheric nor region-specific, and that changes in CBF are unlikely to be a major mechanism of neuroprotection in the acute phase of ischemia in this stroke model.

Although consistent with many established techniques, the accuracy of perfusion measurements by arterial spin labeling could be subject to errors from the magnetization-transfer (Silva *et al*, 1995), transit-time (Zhou *et al*, 2001), and water-exchange (Zhou *et al*, 2001; Parkes and Tofts, 2002) effects. The magnetization-transfer effect was not an issue with the actively decoupled two-coil system (Duong *et al*, 2000). Transit-time effect in small animals and water-exchange effect are small (Zhou *et al*, 2001; Parkes and Tofts, 2002) and unlikely to alter the conclusions of this study. Many of these effects are more severe at low CBF value. Despite these limitations, CBF imaging remains a valuable noninvasive tool for imaging strokes.

It has been postulated that there is a critical CBF threshold(s) below which tissues are destined to become infarcted. Crockard *et al* (1987) used a hydrogen-clearance technique and derived a CBF threshold of 0.2 mL/g min that correlated with the loss of high-energy phosphates in gerbils. Two studies using ^{14}C-iodoantipyrine autoradiography reported a critical CBF threshold of 0.18 and 0.19 mL/g min that corresponded to energy failure at 2 h after permanent MCAO in rats (Hoehn-Berlage *et al*, 1995; Kohno *et al*, 1995). The CBF threshold that approximated lactate acidosis was shown to be 0.31±0.11 mL/g min (Hoehn-Berlage *et al*, 1995), and diffusion disturbance was observed at CBF values below 0.34 to 0.41 mL/g min depending on the duration of ischemia (Kohno *et al*, 1995). Belayev *et al* (1997) and Zhao *et al* (1997) used ^{14}C-iodoantipyrine autoradiography and derived upper CBF limits for the ischemic core and the penumbra of 0.24 and 0.47 mL/g min, respectively; the average penumbral CBF threshold was reported to be 0.3 mL/g min. These findings suggest that different critical thresholds exist for different biological events after ischemia and that these thresholds are dependent on animal models, duration and/or severity of ischemic injury, and the accuracy of cross-modality comparison. While these critical thresholds derived using different postmortem correlation techniques are expected to be slightly different from each other, our TTC-derived thresholds were most consistent with the *onset* of reduced glucose metabolism and lactate acidosis but before energy failure (Hoehn-Berlage *et al*, 1995; Kohno *et al*, 1995), and the penumbral CBF threshold (Belayev *et al*, 1997, Zhao *et al*, 1997).

Other properties of DMSO which may confer neuroprotection include antiinflammatory effects (Wood and Wood, 1975), impairment of platelets aggregation and adhesiveness (Dujovny *et al*, 1983; Schiffer *et al*, 1976), prevention of glutamate-induced excitotoxic death in hippocampal neurons (Lu and Mattson, 2001), Na^+-channel blocking activity (Hulsmann *et al*, 1999), and free radical scavenging (Repine *et al*, 1981). These data show that DMSO is capable of modifying a variety of subsystems believed to be involved in the pathophysiologic cascade of ischemia. It is not yet known which effect is the most prominent and responsible for its neuroprotective properties. It seems more likely that the benefits are the result of a more complex interaction of several mechanisms and the actions on different subsystems deliver additive or synergistic effects.

The most robust effect we observed was when DMSO was administered 20 h before ischemia. The reason for this greater degree of neuroprotection is unclear. Dimethyl sulfoxide has a biologic half-life of 10 to 12 h, and after a single intravenous injection in rats, approximately 80% is eliminated after 24 h (Kolb *et al*, 1967; Denko *et al*, 1967).

Because a low dose of even 0.09 mg/kg DMSO (6% of the dose used in this study) has been shown by others to provide substantial neuroprotection (Shimizu *et al*, 1997), the efficacy of a 20 h pretreatment may be the result of residual DMSO in the brain tissue. Alternatively, indirect mechanisms initiated by DMSO pretreatment, such as induction of gene expression (Rifkind *et al*, 1983), may also contribute to the observed effects.

Our data suggest DMSO should be revisited as a candidate for stroke therapy. It was effective in a reasonable time window in this permanent occlusion model and showed long-lasting neuroprotection. Dimethyl sulfoxide exerted its effect by preservation of the diffusion/perfusion mismatch, an approximation of the tissue at risk with clearly limited lifespan, and the main target for rtPA thrombolysis (Schlaug *et al*, 1999). However, complete recanalization within the first hours after rtPA treatment has been reported to occur in only about one third of the patients (Christou *et al*, 2000; Chalela *et al*, 2004; Rother *et al*, 2002). Thus, a drug capable of protecting the mismatch tissue for several hours, such as DMSO, could extend the time window for eventual recanalization. Dimethyl sulfoxide may also offer an option as a preventative measure in patients undergoing procedures with an increased risk of developing perinterventional brain ischemia, such as carotid stenting or coronary artery bypass surgery. Dimethyl sulfoxide treatment has been shown to be feasible and safe over several days in case series of acute stroke patients (Karaca *et al*, 2002) and patients with increased ICP (Karaca *et al*, 1991; Waller *et al*, 1983), at doses comparable with those used in this study (1.12 g/kg/day and 1 to 1.12 g/kg every 6 h, respectively). Adverse side effects including hypernatremia, fluid overload, and hemolysis have mainly been reported at high rates of infusion or highly concentrated solutions (Waller *et al*, 1983; Marshall *et al*, 1984).

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