Vasomotor Effects of Dimethyl Sulfoxide on Cat Cerebral Arteries In Vitro and In Vivo

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SUMMARY We studied the direct vascular effects of dimethyl sulfoxide (DMSO) in isolated middle cerebral arteries and on pial arteriolar caliber after subarachnoid perivascular microinjection in chloralose-anesthetized cats, and on brain retraction in cats given DMSO intravenously. DMSO did not constrict isolated cerebral arteries at any of the concentrations studied (10^-10 to 4 x 10^-1 M). In middle cerebral arteries precontracted with potassium, 5-hydroxytryptamine, prostaglandin F2 alpha, or with mechanically raised tone, DMSO at concentrations of 10^-10 to 10^-2 M had no significant effects; at concentrations greater than 10^-2 M, DMSO consistently relaxed the arteries, probably because of the hyperosmolarity of the bathing solution. Microapplication of DMSO (10^-10 to 10^-2 M) around pial arterioles on the cortical surface did not change arteriolar caliber significantly. Higher concentrations of DMSO (1%) increased arteriolar caliber by 56 ± 4% (p < 0.001), probably as a consequence of solution hypertonicity. DMSO did not modify in vivo cerebrovascular responses to alterations in perivascular potassium ion concentrations. Intravenous administration of DMSO did cause obvious brain shrinkage. These data provide no support for the view that direct cerebral vascular effects play a major role in the clinical efficacy of DMSO, but are consistent with the hypothesis that DMSO's ability to lower intracranial pressure derives from its osmotic effect on cerebral issue.

DIMETHYL SULFOXIDE (DMSO) has been reported to reduce morbidity and mortality in some1,2 but not other studies3,4 of cerebral infarction, pressure-induced ischemia,5 and trauma,6,7 and to lower traumatic intracranial hypertension and improve cerebral perfusion in humans8,9 and animals.7,10,11 In our current clinical trial of the effect of DMSO on raised intracranial pressure (ICP) after severe head injury, ICP was lowered within minutes after intravenous (IV) administration of DMSO in some patients (unpublished data). The mechanism of action of DMSO is unknown, although hypertonicity and direct cerebrovascular effects are two possible hypotheses. In this study, we investigated both the direct vasomotor effects of DMSO on isolated feline middle cerebral arteries in vitro and alterations resulting from microapplication of DMSO around individual pial arterioles on the cortical surface of anesthetized cats.

Materials and Methods

Response to Middle Cerebral Pial Arteries in Vitro

These experiments were conducted using techniques described previously.12 Six cats were anesthetized with 5% halothane, exsanguinated, and decapitated. The middle cerebral arteries were dissected and placed in cold Krebs'-Ringer's buffer solution (composition in mM: NaCl, 118; KCl, 4.5; MgSO4·7H2O, 1.0; KH2PO4, 1.0; NaHCO3, 25.0; CaCl2·2H2O, 2.5; glucose, 6.0). Sections of artery 5 to 6 mm long were mounted on two L-shaped metal holders, one of which was attached to a force-displacement transducer to allow attachment to the vessel and the buffer solution was changed every 15 minutes. After a 15-minute period during which no further adjustments were needed to maintain the 400-dyne load, each vessel was tested with prostaglandin F2 alpha (PGF2 alpha, 2.5 μM). Vessels that did not constrict were excluded from further study. To accentuate the vasodilatory properties of DMSO, vessel tone was increased in some experiments by adding PGF2 alpha (2.5 μM) or 5-hydroxytryptamine (5-HT, 0.3 μM) to the buffer, or by replacing the NaCl in the Krebs'-Ringer's solution with KCl to obtain a potassium concentration of 127 mM.

Log concentration-response curves were obtained by cumulative addition of DMSO to achieve bath concentrations of 10^-10 to 4 x 10^-1 M (about 7.5 x 10^-10% to 3% DMSO by volume). DMSO concentrations that produced the half maximal response (EC50) were calculated, as were the maximal responses (EAmax) in dyne, for vessels with mechanically or pharmacologically increased tone.

Vasomotor Response of Pial Arterioles in Vivo

These investigations were performed using a previously described technique.13 Thirteen cats of either sex weighing 2 to 5 kg were anesthetized with an IV...
infusion of alphaxalone, 9 mg/kg, and alphadolone, 3 mg/kg (Saffan, Glaxovet Ltd., Middlesex, England), intubated, and ventilated by intermittent positive pressure with a 33% N2O/67% O2 mixture. Anesthesia was maintained by periodic IV infusion of a 1% alphachloralose solution. Exhaled gases were monitored continuously with an infrared CO2 analyzer; arterial pO2, pCO2, and pH were measured periodically so they could be maintained within a physiologic range (pO2 = 172 ± 28 torr; pCO2 = 30 ± 2 torr; pH = 7.34 ± 0.04). The head of the cat was placed in a stereotaxic frame, and a 2 × 3-cm left parietal craniotomy was made with a dental drill under continuous saline irrigation. The dura was covered with a 1.5 cm layer of circulating mineral oil (38°C) and then opened under magnification (Bausch & Lomb stereomicroscope with zoom lens) and cold lighting from fiberoptic sources (Schott). Arteriolar calibers were measured by a standard image-splitting technique.13

Shortly before use, DMSO (Rimso, Research Industries, Salt Lake City, Utah) was dissolved to various concentrations (0.0001% to 1%) in normokalemic mock cerebrospinal fluid (CSF) (composition in mM: Na+, 156; K+, 3; Ca++, 2.5; HCO3-, 12; Cl-, 152) or mock CSF with 10-mM K+ (Na+, 149; K+, 10; Ca++, HCO3-, Cl- unchanged) or 40-mM K+ (Na+, 129; K+, 40; Ca++, HCO3-, Cl- unchanged). One percent DMSO was prepared by diluting 1 ml of pure DMSO (1.1 g) to 100 ml by addition of mock CSF. All solutions were adjusted to pH 7.2 by aeration with 5% CO2. The solutions were sterilized with a 0.22-μm filter, and periodically adjusted to document changes in the variance with those induced by injection of mock CSF.

Results

Middle Cerebral Arteries Ex Vivo

At concentrations of 10⁻⁵ to 10⁻² M, DMSO had little effect on the tone generated by middle cerebral arteries (n = 6) in vitro, although it produced slight relaxation at higher concentrations (10⁻¹ to 4 × 10⁻¹ M) (fig. 1). In vessels constricted with PGF2α (620 ± 100 dyne; n = 11), DMSO resulted in vasodilatation equivalent to 85% of the induced tone, but only at the highest concentrations studied. The EC₅₀ was 1.08 ± 0.35 × 10⁻¹ M and the E₅₀ was 490 ± 70 dyne. 5-HT caused cerebral artery constriction amounting to 890 ± 150 dyne (n = 7). The subsequent cumulative addition of DMSO resulted in vasodilatation equal to 60% of the induced tone; again, this effect occurred only at the highest concentrations. The EC₅₀ was 0.94 ± 0.70 × 10⁻¹ M and the E₅₀ was not different from that in vessels constricted with PGF2α (510 ± 70 dyne). The buffer solution containing 127 mM potassium provoked maximal vasoconstriction of the cerebral vessels (150 ± 300 dyne; n = 13). Once again, DMSO relaxed middle cerebral arteries, but only in high concentrations (>10⁻² M); the amount of relaxation (13% of the induced tone) was somewhat less than in arteries preconstricted with PGF2α or 5-HT. However, the EC₅₀ value was similar to that described above (1.56 ± 0.36 × 10⁻¹ M), and the E₅₀ was 230 ± 50 dyne.

Pial Arterioles in Vivo

Perivascular microinjections of artificial CSF had little effect on pial arteriolar caliber (mean response, 1.5 ± 2.4%; n = 18). In artificial CSF, DMSO in concentrations of 0.0001% to 0.5% produced no significant effect on arteriolar caliber, although at high concentrations (e.g., 1%), arteriolar caliber was increased significantly (fig. 2). Neither the cerebrovascular dilatations provoked by microinjections of moderately elevated potassium concentrations (10 mM) nor the constrictions provoked by microinjections of markedly elevated potassium concentrations (40 mM)

FIGURE 1. Dilating effect of increasing dimethyl sulfoxide (DMSO) concentrations in annular segments of cat middle cerebral artery preconstricted by mechanical loading or by addition of potassium, prostaglandin F2α (PGF2α), or 5-hydroxytryptamine (5-HT).
were modified by the presence of DMSO (0.1%, or $10^{-2}$ M) in the injectate (fig. 3).

**Osmolar Effects**

The mean osmolality of the Krebs' solution — 285 mosmol/l, determined by freezing point depression — was not altered by DMSO in concentrations of $10^{-10}$ to $10^{-2}$ M, but it was increased significantly by concentrations greater than $10^{-2}$ M (fig. 4). This change in osmolality correlated extremely well with the concentration at which vasodilatation was first observed in the cerebral vessels.

The presence of DMSO in concentrations less than 0.01% had no effect on the osmolality of the mock CSF, but higher concentrations produced concentration-dependent increases in osmolality (e.g., artificial CSF: 299 ± 4 mosmol; DMSO 1%: 486 ± 5 mosmol). To determine the contribution of hypertonicity to the vascular effects of DMSO in vivo, hypotonic solutions of CSF (454 mosmol) produced by increasing the NaCl concentration were administered perivascularly.

**Intravenous Infusion of DMSO**

After the last perivascular microinjection in 2 cats early in the series, intravenous infusion of 10% DMSO (1 g/kg) caused the brain to shrink visibly and recede from the edge of the dura. Later, in another 2 cats, we measured the changes in brain surface location and mean arterial pressure (MAP) during infusion of DMSO. In the first cat, the brain retracted 0.7 mm within 15 minutes after the start of the infusion (0.25 g/kg DMSO delivered) and remained retracted even though blood pressure rose from 75 to 100 mm Hg. Within 2 minutes after a second dose of DMSO, given more rapidly, MAP rose from 75 to 138 mm Hg, and was accompanied by a slight transient increase in brain size (maximal retraction, 0.4 mm). Ten minutes after the second injection, the brain retracted further, to a maximum of 1.9 mm, despite continuing hypertension (MAP = 110 mm Hg). In the second cat, the brain retracted 1.1 mm within 10 minutes after the start of the infusion (0.16 g/kg DMSO delivered) and remained retracted even though blood pressure rose from 75 to 100 mm Hg. Within 2 minutes after a second dose of DMSO, given more rapidly, MAP rose from 75 to 138 mm Hg, and was accompanied by a slight transient increase in brain size (maximal retraction, 0.4 mm). Ten minutes after the second injection, the brain retracted further, to a maximum of 1.9 mm, despite continuing hypertension (MAP = 110 mm Hg). In the second cat, the brain retracted 1.1 mm within 10 minutes after the start of the infusion (0.16 g/kg DMSO delivered), even though MAP increased from 112 to 127 mm Hg. At 1 hour, retraction was 2.1 mm despite hypertension to 160 mm Hg. The second DMSO injection further retracted the brain to a maximum of 2.9 mm when the MAP was 125 mm Hg. Postinjection hypertension to 190 mm Hg caused a slight increase in brain size, but even then the brain surface was 1.2 mm below its preinfusion position.
Discussion

In light of reports that DMSO lowered ICP rapidly in monkeys with experimental brain compression and missile injury, and prevented infarction in dogs subjected to middle cerebral artery occlusion and embolism, a limited clinical trial was conducted in patients with severe head injury. DMSO successfully reduced traumatic intracranial hypertension resistant to mannitol, hyperventilation, and barbiturate coma. In experimental brain injury models and in head injury patients at our institution (unpublished data) and elsewhere, DMSO has lowered ICP, sometimes within minutes. The mechanism by which DMSO lowers ICP is unknown, but the rapid onset of action raises the possibility of direct cerebral vasoconstriction and consequent reduction in intracranial volume and pressure.

In our experiments, however, DMSO did not cause cerebral vasocostriction in vivo or in vitro at any concentration studied. In vitro, DMSO in concentrations at or above 0.2% produced significant and progressively greater vessel dilation; in vivo, vessel dilation occurred only with 1% DMSO solutions. These concentrations might be achieved transiently in the blood during IV DMSO infusion, but because of its ability to cross tissue membranes, DMSO distributes rapidly throughout the body, and clinical and experimental doses used probably yield DMSO tissue concentrations of 0.1% to 0.3% or less. Thus, the DMSO concentrations that produced arterial dilatation in our study probably exceed clinically achievable levels.

DMSO did exert significant relaxant effects on the cerebral vasculature both in vitro and in vivo, but only at concentrations sufficient to elevate the osmolality of the organ bath or the injectate. Hyperosmolarity in blood and tissue causes vasodilatation in the peripheral circulation (muscle, gut, skin), and Wahl et al. found that pial arteriolar caliber increases significantly when exposed to perivascular osmolarity above 317 mosmol. Our results confirm pial artery vasodilatation with DMSO hyperosmolarity, and even greater vasodilatation with NaCl hyperosmolarity. Rapid tissue permeation by DMSO allows it to cross cell membranes so that the osmotic gradient across the arterial smooth muscle cell wall is smaller for a given extracellular osmolality produced by DMSO than by NaCl. Human red blood cells exposed to extracellular DMSO are initially subjected to an osmotic gradient across the cell membrane. However, DMSO begins to penetrate the cells within 1 minute and osmotic equilibrium is established within 15 minutes.

We have shown that systemic infusion of DMSO in doses comparable to those used in humans produced rapid brain shrinkage in cats. Although some autoregulatory arterial constriction accompanying hypertension might have accounted for a small portion of this shrinkage, retraction was greatest when blood pressure was nearest to control, which suggests a true loss of brain volume that was not caused by alterations in blood volume. DMSO infusions have been shown to increase cerebral blood flow, and intravascular blood volume, which would argue against decreased cerebral blood volume as the cause of brain shrinkage in our experiments. The time course of brain surface retraction was similar to that reported for ICP reduction from both DMSO and urea in monkeys and in our preliminary studies in humans.

The possibility that the cerebrovascular responses of the cat differ substantially from those of man cannot be absolutely excluded, but it seems highly unlikely. The responses of human and feline cerebral arteries to a wide variety of agents, including noradrenaline, serotonin, histamine, prostaglandins, neuronal polypeptides, and calcium antagonists, are qualitatively and quantitatively similar. Nor does DMSO show striking interspecies differences in toxicity. Thus, the results of these studies in cats probably apply to DMSO use in humans.

In conclusion, DMSO exerted no direct vascular effect that would contribute to its ability to lower ICP, although cerebrovascular dilatation consistent with an osmotic effect occurred at high concentrations of the drug that would be unlikely to occur clinically. Nonetheless, systemic administration of DMSO in cats produced brain shrinkage, which is consistent with its reported ability to lower ICP. These observations emphasize the need for further investigation of the mechanisms of action of DMSO, but its lack of potentially deleterious actions on the cerebral vasculature and its ability to lower ICP justify continued use of DMSO in rigorously controlled studies of human head injury.

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