Failure of Standards in Reporting the Composition of Artificial Cerebral Spinal Fluid in Studies of the Pial Blood Vessels

To the Editor:

Reproducible experimental results require the replication of the experimental conditions under which the experiment was first performed. Relevance to humans may depend on the degree to which those conditions resembled those to which humans are exposed. With respect to studies of vascular reactivity, a most important parameter is the composition of the fluid bathing the vessel. For vessels on the brains surface, so-called pial vessels, this fluid is the cerebrospinal fluid (CSF). Experiments studying these vessel, in vivo or in vitro, generally bathe the vessels in an artificial CSF, sometimes called a “mock” CSF, or some other fluid, sometimes called a physiological salt solution and sometimes simply a buffered solution. A question that should immediately arise, if relevance to humans is an issue, is whether the fluid used has the composition of cerebrospinal fluid, and if not, is it the composition of the experimental species’ CSF or of human CSF. At present there is no data to tell us whether or not it would be better to always replicate the composition of human CSF, even if this differs from that of the animal in use, or whether, instead, the mock CSF should resemble that of the animal itself. CSF contains urea, but not everyone adds urea to the artificial CSF, and there are no studies showing the effect of the urea on the vascular responses. Indeed there is virtually no information on the effect of altering one or more aspects of CSF composition, other than pH and changing the concentration of one ion or another so that it is clearly out of mammalian physiological range. Moreover, recent studies show that under some circumstances, when a mock CSF flows over the pial surface in a continual suffusion, some vascular responses may be dramatically altered because essential substances, not thought of as part of CSF but in the tissue and its extracellular space, may be washed away. In the absence of the requisite studies, this letter will not address the question of “which formulation of mock CSF is better—human or animal?” Nor will it address the importance, or lack thereof, of the addition of urea or of the presence or absence of continuous rapid suffusion. It will, however, direct itself to the most basic requisite for a scientific presentation—the need to describe the fluid one is using so others may replicate your experimental conditions.

Unfortunately, with respect to studies concerning cerebrovascular reactivity in vivo, our leading journals and, presumably, others have demonstrated a dramatic failure to maintain the standards displayed for studies of other vascular beds. Why this is so cannot be stated. But that it is so is evident from the following analysis.

For studies of pial vessels, 3 journals were consulted over the past 4 years. These journals were Stroke, The Journal of Cerebral Blood Flow and Metabolism, and The American Journal of Physiology (AJP). As a “control,” papers concerning extracerebral vascular beds were surveyed over the same time period in AJP and in Microcirculation. A total of 34 in vivo studies of extracerebral vascular reactivity were located. Of these, only 2 (6%) failed to specify the composition of the bathing fluid. In striking contrast, of the 49 studies of pial vessels, 21 (43%) failed to specify the composition of the bathing solution. In only 3 of these 21 articles was there even a reference to an earlier paper that described the bathing solution. In some of the remaining 18 cases a reference to the preparation as a whole was given, but perusal of that reference failed to give the composition of the bathing fluid.

In the 18 papers without even a helpful reference (37% of the total in 49 studies), an effort was made to determine what the composition of the fluid might be, by locating past papers of the authors. Sometimes, the first description of the fluid was found in a paper published many years earlier in conjunction with some other author. In the absence of a reference to the earlier paper, it seemed unwise to assume that the composition of the fluid used in the recent year was identical to that described in the much earlier paper.

The absence of a description of the composition of the fluid was even more glaring than suggested by the simple fact that in 18 of 49 papers there was neither description nor a useful reference to the composition of the fluid. That is because of the 31 papers which did give a description, 8 came from a single laboratory. In other words some authors publish a lot and consistently provided essential experimental details and thus bias the statistics toward the side of compliance with a necessary standard.

Finally, when the composition of the irrigating solution is provided, it is necessary to consider the units in which it is presented. The preferred units would be in molar or millimolar terms or in terms of milliequivalents. If, instead, the grams or milligrams per liter of each substance are given, it falls to the reader to make the required calculation.

It should be clear from this analysis that journal editors, reviewers, and authors have been delinquent in meeting a reasonable standard for the publication of in vivo studies of pial blood vessels, a standard that is almost always met when other vessels and/or in vitro studies are the subject of the reports, and a standard that is frequently violated for pial vessels even when it is met for other vascular beds in the very same journal. It is hoped that these remarks will lead to correction of that situation.

William L. Rosenblum, MD
Department of Pathology
Medical College of Virginia
at Virginia Commonwealth University
Richmond, Virginia

Lipoprotein(a) and the Risk of Stroke

To the Editor:

Despite a number case-control studies in the literature suggesting that lipoprotein(a) is a risk factor for cerebrovascular disease, both the recent article by Glader et al and the report from the Physicians Health Study by Ridker et al failed to demonstrate an association of lipoprotein(a) and the future risk of stroke. One possible explanation discussed by Glader et al is the paucity of young stroke patients in their study population. Nagayama et al had previously reported significantly higher lipoprotein(a) levels in patients under age 45 years with atherosclerotic stroke. In the Stroke Prevention in Young Women Study, we examined the role of lipoprotein(a) in a population-based study of women under the age of 45 years with ischemic strual of a both usual and multivariate analyses, we found no significant difference in lipoprotein(a) levels between cases and age-matched controls. Even among the subset of patients with atherosclerotic stroke, we could not demonstrate an association

William L. Rosenblum, MD
Department of Pathology
Medical College of Virginia
at Virginia Commonwealth University
Richmond, Virginia
with elevated lipoprotein(a) levels. The number of patients in our study with atherosclerotic stroke was small (n = 15), however, but comparable to that of Nagayama et al (n = 11).

The structure of lipoprotein(a) and its potential link to both atherosclerosis and hypercoagulability is an intriguing hypothesis, but at this time the epidemiologic basis for a relationship of lipoprotein(a) and stroke remains uncertain.

Robert J. Wityk, MD
Department of Neurology
Johns Hopkins Hospital
Baltimore, Maryland

Steven Kritter, MD
Department of Neurology
and Department of Epidemiology and Preventive Medicine
University of Maryland at Baltimore
Geriatrics Research, Education, and Clinical Center
Baltimore Department of Veterans Affairs Medical Center
Baltimore, Maryland


Intrathecal Sodium Nitroprusside Improves Cerebral Blood Flow and Oxygenation in Refractory Cerebral Vasospasm and Ischemia in Humans

To the Editor:

With great interest we read the article by Thomas et al recently published in Stroke. The authors proposed the intrathecal administration of the nitric oxide donor sodium nitroprusside (SNP) as a novel means to treat refractory cerebral vasospasm. However, although amelioration or even reversal of large cerebral vessel constriction was shown by cerebral angiography, the article failed to provide data on the acute and long-term effect of SNP on cerebral vasospasm–associated ischemia and hypoxia. Therefore, in addition to their report, we here show the effect of intrathecal SNP on episodes of severely reduced cerebral blood flow and brain tissue oxygenation in a patient suffering from refractory cerebral vasospasm.

Multimodality Neuromonitoring During Intrathecal Treatment With SNP

<table>
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<tr>
<th></th>
<th>MABP, mm Hg</th>
<th>ICP, mm Hg</th>
<th>CPP, mm Hg</th>
<th>rCBF, ml/100 g/min</th>
<th>ptiO2, mm Hg</th>
</tr>
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<tbody>
<tr>
<td>During HHH therapy</td>
<td>141</td>
<td>16</td>
<td>125</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Before delivery of 20 mg SNP*</td>
<td>111</td>
<td>15</td>
<td>96</td>
<td>3</td>
<td>2</td>
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<tr>
<td>30 min after 20 mg SNP ICV</td>
<td>102</td>
<td>21</td>
<td>82</td>
<td>12</td>
<td>15</td>
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<tr>
<td>3 h after 20 mg SNP ICV†</td>
<td>107</td>
<td>13</td>
<td>94</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>30 min after additional 16 mg SNP ICV</td>
<td>97</td>
<td>12</td>
<td>85</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>24 h after additional 16 mg SNP ICV</td>
<td>103</td>
<td>15</td>
<td>88</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>48 h after additional 16 mg SNP ICV</td>
<td>106</td>
<td>23</td>
<td>83</td>
<td>17</td>
<td>23</td>
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All parameters were sampled continuously at the bedside at 1 Hz. For each time point represented, the values were averaged over 50 seconds. Severe ischemia and hypoxia were defined as rCBF < 6 ml/100 g/min and ptiO2 < 10 mm Hg. ICV indicates intracerebroventricular administration.

*After discontinuation of HHH therapy; †prior delivery of additional 16 mg SNP.

Seven days after subarachnoid hemorrhage (Hunt and Hess grade II) and uneventful operation of bilateral middle cerebral artery (MCA) aneurysms, a 31-year-old female patient presented with right-sided hemiparesis and progressive neurological deterioration. Transcranial Doppler recordings of the MCAs had elevated from values <100 cm/s to 205 cm/s and 180 cm/s on the left and right sides, respectively. Cerebral angiography revealed severe cerebral vasospasm, which was most pronounced in the left MCA (M1 and M2 segments). Consequently, the patient was sedated and intubated, and a right frontal ventriculostomy for intracranial pressure (ICP) monitoring was performed. In addition, a polarographic brain tissue oxygen (ptiO2) microprobe2 and a thermal diffusion regional cerebral blood flow (rCBF) microprobe3 were implanted subcuticularly into the white matter of the left frontal MCA territory. Thereby, using a PC-based multimodality neuromonitoring system, mean arterial blood pressure (MABP), ICP, cerebral perfusion pressure (CPP = MABP – ICP), ptiO2 and rCBF were monitored continuously at the bedside with a sampling rate of 1 Hz. Thresholds for severe ischemia and hypoxia were defined as rCBF < 6 ml/100 g/min and ptiO2 < 10 mm Hg, as previously reported.3,4

Following the diagnosis of cerebral vasospasm, selective intra-arterial administration of papaverin (300 mg), hypertensive/hypervolemic/hemodilutional (HHH) therapy, and increasing the inspired fraction of oxygen to >70% were initially successful in controlling cerebral ischemia and hypoxia (Table). When HHH therapy, however, had to be discontinued due to cardiac decompensation of the patient, rCBF and ptiO2 dropped again to severe ischemic and hypoxic values (Table). As a last measure, SNP was delivered intraventricularly via the ventriculostomy, as previously described1 (4 mg/mL, dissolved in the patient’s cerebrospinal fluid). Dosing of SNP was intermittent and adjusted to changes in rCBF and ptiO2. Despite a modest reduction in CPP, rCBF and ptiO2 rapidly increased 4-fold and 7.5-fold, respectively, within 30 minutes after delivery of 20 mg SNP (Table). Because this effect was only transient and all parameters had returned to baseline 3 hours after treatment, an additional 16 mg of SNP was administered. Similar to the first SNP administration, rCBF and ptiO2 values rapidly improved again within 30 minutes after delivery (Table). The second SNP administration, however, resulted in a permanent reversal of severe cerebral ischemia and hypoxia, eventually achieving moderate clinical recovery of the patient.

In the present report we demonstrate for the first time that the intrathecal administration of the nitric oxide donor SNP is effective in improving cerebral blood flow and oxygenation in otherwise refractory cerebral vasospasm in humans. The potentially temporary efficacy and possible adverse effects on CPP and ICP, however, warrant a continuous bedside multimodality neuromonitoring of hemodynamic parameters, cerebral blood flow and brain tissue oxygenation during SNP therapy.
This study was supported by the German Research Foundation (VA 151/5-1)

Peter Vajkoczy, MD
Ulrich Hubner, Dr Ing
Peter Horn, MD
Christian Bauhuf, MD
Claudius Thome, MD
Lothar Schilling, MD
Peter Schmiedek, MD

Department of Neurosurgery
Klinikum Mannheim
University of Heidelberg
Mannheim, Germany


Response

Dr Vajkoczy and colleagues present an intriguing observation that appears to have been beneficial to the patient. Although we are cautious in interpreting these limited experiences in terms of efficacy, these results corroborate our clinical experience with intrathecal SNP in several ways. (1) As illustrated in our recent article1 referenced by these authors, improvement in CBF2 was suggested by acceleration of cerebral circulation time at the same MABP and ICP. This sometimes occurred without a dramatic change in the caliber of cerebral conductance vessels and was interpreted by us as evidence of dilated collateral circulation below the level of angiographic resolution. The same inference, i.e., increased regional cerebral blood flow, applies to the enhanced angiographic blush observed in treated patients (Figure 1). (2) MAB remained relatively stable, and ICP remained within normal range. (3) The improvement in measured parameters was accompanied by clinical improvement, suggesting minimal or no neurotoxicity. Indeed our most recent experience with treating patients by this method in the intensive care unit without angiography has been noteworthy for several episodes of profound and rapid neurological improvement within several hours of treatment, sometimes without a clear relationship to the vessels deemed constricted by transcranial Doppler (J. Thomas et al, unpublished observations). Such observations also suggest an effect at the level of collateral circulation. (4) The effect of the first treatment by these authors was transient, and vasospasm returned, supporting the hypothesis that it is indeed a substrate-dependent phenomenon that is liable to recur until the substrate (oxyhemoglobin1) is exhausted.2

It is also interesting that the reported patient was initially treated with intra-arterial papaverine, the effect of which was not lasting. This has also been our experience with papaverine.

Finally, a caveat regarding conclusions drawn from relatively few patients is in order. Our purpose in the referenced article was to emphasize the apparent safety of intrathecal SNP in initial trials, and not its efficacy. It does appear that the treatment may be efficacious in some patients, even dramatically so, but we have seen two patients whose established vasospasm has not responded to this treatment, and the reasons for this are still unclear. Several points are worth mentioning in this regard. (1) Dosage must be scrupulously controlled to avoid hypotension, but nitric oxide may be rapidly absorbed by the hemoglobin in the subarachnoid clot, thereby making elevated dosage not only possible but necessary. This problem of a hemoglobin “sink” might theoretically be aggravated by intraventricular hemorrhage if the preparation is being administered intraventricularly. (2) The relatively slow rate of infusion of very small volumes of SNP makes photo-inactivation of the compound a very serious threat. For this reason even the smallest gap in light protection (foil wrap) must be avoided. This risk cannot be overemphasized. (3) It has been our experience that if vasospasm severity exceeds a certain threshold, it may be impossible to reverse with this treatment. For this reason we begin treatment immediately after treatment.

Several additional treatments were required in the intensive care unit over several days. The patient made a full neurological recovery and has returned to work as a computer systems analyst.

Figure 1. Left ICA AP cerebral angiogram of a 40-year-old man presenting with delayed neurological deficit after aneurysmal subarachnoid hemorrhage. The patient developed dysphasia and right hemiparesis. The most pronounced angiographic effect was not dramatic dilation of constricted large conductance vessels, but of small collaterals resulting in enhanced angiographic blush (arrows) and decreased cerebral circulation time (indicated in figure). Blood pressure and ICP were unaffected by treatment. A, before treatment; B, immediately after treatment. Several additional treatments were required in the intensive care unit over several days. The patient made a full neurological recovery and has returned to work as a computer systems analyst.
very early, preferably as soon as the aneurysm is secured for a patient at high risk for vasospasm, and upon obtaining any evidence of impaired cerebral circulation, such as transcranial Doppler or cerebral blood flow or oximetry, even when the patient remains neurologically stable. (4) As mentioned in our article, intracranial pressure problems may make treatment by this method untenable, at least in the short term, if not definitively. Thus severe vasospasm may become established. Such patients should be considered early for balloon angioplasty if possible, despite its restriction to the larger vessels. (5) Treatment may be hindered by nausea and vomiting, which seems to be a problem only in fully awake patients. High-dose ondansetron-HCl, which works synergistically with dexamethasone (3), is very helpful in this regard. (6) Since ventriculostomy is a “blind” neurosurgical procedure, attention must be given to correct intraventricular catheterization before treatment is begun. CT should be obtained initially, but safety is ultimately determined by the facile withdrawal of cerebrospinal fluid from the catheter just prior to treatment. Although an intraparenchymal injection of 1.0 mL may not be harmful in a patient without intracranial hypertension (see Figure 2), such a patient may have no benefit from the treatment administered extra-ventricularly. (7) The role of cerebrospinal fluid circulation in the successful administration of intrathecal sodium nitroprusside remains uncertain. Such circulation is obviously impaired in many patients with high-grade aneurysmal subarachnoid hemorrhage.

We applaud the authors for their meticulous and successful care of their patient, and for their careful and encouraging measurements of treatment effect. We emphasize, however, that the true efficacy of this treatment remains unknown, and only through a methodical prospective analysis can its value be determined. We look forward to reporting the results of our prospective study in the very near future.

Jeffrey E. Thomas, MD
Division of Cerebrovascular Surgery and Interventional Neuroradiology,
Department of Neurological Surgery
Thomas Jefferson University and Wills Neurosensory Institute
Philadelphia, Pennsylvania

Letters to the Editor

Figure 2. Electron microscopic appearance of rat cerebral cortex 7 days after intraparenchymal and intraventricular administration of SNP in concentration identical to that in human patients (4.0 mg/mL). The ultrastructural architecture is preserved; synaptic vesicle and cell membranes are intact (Erol Veznedaroglu, MD, unpublished data). Magnification ×30,000.

The Relative Contributions of the Anterior and Posterior Circulations to Global Cerebral Blood Flow

To the Editor:

We read with interest the report by Scheel and colleagues1 of the duplex ultrasound derived volumetric flows of the anterior and posterior components of the cerebral circulation published in Stroke. In discussing their main finding that the anterior circulation contributes 76% and the posterior circulation 24% to the total global cerebral flow, we authors noted that there were no other reports available for comparison.

We published a report in 19952 of our findings from a similar investigation in a series of 21 healthy adults describing the relative flow contributions of the anterior and posterior circulation to the total global cerebral blood flow. We found that the anterior and posterior circulations contributed 82% and 18%, respectively, to total global cerebral blood flow, which is in quite close agreement with the findings of Scheel and colleagues. We also noted that these relative flow contributions were independent of gender and in precise concordance with the expected blood flow distribution based on the differences in the measured internal lumen diameters of the internal carotid arteries and vertebral arteries. The absolute values, however, for the volumetric flow rates differed between the 2 studies, with our reported values uniformly lower by about 40%, despite the use in both studies of a similar time-averaged velocity calculation throughout the cardiac cycle of all particles within the sample volume overlaying the entire lumen. Our report discussed the numerous potential causes and sources of systematic error, and such considerations in combination with the lack of a gold standard by which to determine absolute or actual flow suggests to us that duplex-derived direct flow measurements be used as indices of flow magnitude rather than true determinations of actual flow. We agree with the authors that a reliable, noninvasive index of regioselective blood flow could provide clinically relevant information to benefit patients in the intensive care unit. Improvements in the understanding and measurement of all components of the cerebral circulation should particularly improve validation and monitoring of pharmacological and endovascular therapeutics for stroke.

Robert A. Boyajian, MS, MD
Division of Neurology
Department of Medicine

Shirley M. Otis, MD
Department of Medicine
Division of Neurology
Vascular Laboratory
Scripps Clinic
La Jolla, California

References


Response

We regret that the publication of Boyajian et al. escaped our notice—just as the paper we published a year before that apparently eluded theirs. In 1994 we first reported on global cerebral blood flow volume examinations in 48 healthy adults (mean age 35±12 years), where we found a mean contribution of 76% by the anterior and of 24% by the posterior circulation. Global cerebral blood flow volume was 701±104 mL/min, corresponding to a cerebral blood flow of 50–54 mL/100 g/min (assuming a mean adult brain weight of 1300–1400 g). In a study on children and adolescents, the mean contribution of the posterior circulation to global cerebral blood flow volume declined significantly from 31% at the age of 4 years to 24% at the age of 18 years. During the same period, cerebral blood flow volume decreased significantly (3–9.9 years, 821±116 mL/min; 10–18 years, 727±106 mL/min).

In a recent interobserver study, the reproducibility of global cerebral blood flow volume measurement was shown to be comparable to that of global cerebral blood flow measured with $^{15}$O-labeled water positron emission tomography. In this study, the ratio of anterior to posterior circulation flow volumes was already estimated to be 76% to 24% by both examiners. In the article discussed here, the mean contribution of the posterior circulation to cerebral blood flow volume remained constant (24%) from 20 to 85 years of age, whereas global cerebral blood flow volume decreased from 730±87 mL/min (age group 20–39 years) to 603±106 mL/min (age group 60–85 years).

The flow volumes measured by Boyajian et al. in the internal carotid arteries on both sides (147 and 143 mL/min, respectively) and in the vertebral arteries (34 and 32 mL/min, respectively) of healthy young adults are much lower than those found by our group. They add up to a mean global cerebral blood flow volume of 356 mL/min, which would correspond to a very low global cerebral blood flow of 25–27 mL/100 g/min. Most publications on normal cerebral blood flow in healthy adults, however, report reference data of ~50 mL/100 g/min. We can explain this difference only with a systematic error of flow volume measurement. In contrast to Boyajian and Otis, we are convinced that cerebral blood flow volume can be measured quantitatively, as long as a meticulous examination technique is observed. In a first attempt to pave the way for using this method in clinical routine, we studied cerebral blood flow volume in patients with vascular dementia, where a marked decrease of cerebral blood flow volume was found. At present we are in the process of establishing bedside cerebral blood flow volume monitoring in neurointensive care patients. We would like to encourage neurologists, child neurologists, and neurosurgeons to learn and apply this technique, because we firmly believe in the great potential benefits of the clinical and scientific application of this method.

Martin Schöning, MD
Peter Scheel, MD
Department of Child Neurology
Children’s Hospital of the University of Tübingen
Tübingen, Germany

Failure of Standards in Reporting the Composition of Artificial Cerebral Spinal Fluid in Studies of the Pial Blood Vessels
William I. Rosenblum

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