Effects of a Single Dose of Dantrolene in Patients With Cerebral Vasospasm After Subarachnoid Hemorrhage
A Prospective Pilot Study

Susanne Muehlschlegel, MD, MPH; Guy Rordorf, MD; John Sims, MD

Background and Purpose—New therapies for cerebral vasospasm after subarachnoid hemorrhage are needed because of its high morbidity and mortality rates. We investigated the feasibility and safety of a single dose of intravenous dantrolene and its effect on transcranial Doppler in cerebral vasospasm after subarachnoid hemorrhage.

Methods—In a prospective, open-label, single-dose ascending safety trial, 5 patients received intravenous dantrolene 1.25 mg/kg and the next 5 patients received 2.5 mg/kg over the course of 60 minutes. All other infusions were kept steady and hemodynamic parameters were recorded. Transcranial Doppler was performed at 0, 45, 90, and 135 minutes relative to infusion start. Basic chemistries, serum osmolality, arterial blood gas, and liver enzymes were measured before and after.

Results—Laboratory values and hemodynamic parameters remained unchanged except for a decrease in the systolic blood pressure in the low-dose group (−8 mm Hg; 95% CI, −26 to 10 mm Hg; P=0.027). After correcting for this decrease in blood pressure, peak systolic transcranial Doppler velocities decreased significantly (−26 cm/s; 95% CI, −47 to −5 cm/s; P=0.02), with a borderline change in mean velocities in the low-dose group (−16 cm/s; 95% CI, −36 to 4 cm/s; P=0.07) and peak systolic transcranial Doppler velocity in the high-dose group (−26 cm/s; 95% CI, −56 to 5 cm/s; P=0.05).

Conclusions—In this pilot study, a single dose of intravenous dantrolene in cerebral vasospasm after subarachnoid hemorrhage appears feasible while inhibiting vasoconstriction in the low-dose group, but it may lower blood pressure. Our study provides useful data for the design of larger future studies.

Clinical Trial Registration—URL: http://clinicaltrials.gov. Unique identifier: NCT00964548. (Stroke. 2011;42:00-00.)

Key Words: calcium □ cerebral vasospasm □ dantrolene □ neurocritical care □ ryanodine receptor □ subarachnoid hemorrhage □ vasoconstriction

Subarachnoid hemorrhage (SAH) is one of the most devastating forms of stroke affecting primarily young patients before the age of 65 and has a fatality of 50% in the first 30 days.1, 2 For survivors of the initial insult, cerebral vasospasm (CVSP) is the leading cause of disability and death;3, 4 therefore, it points to an inviting therapeutic target. CVSP occurs in 70% of patients with SAH, and one-third have neurological deficits develop.4 Available treatments for CVSP are limited, consisting of hypervolemic hypertensive therapy and cerebral angiography with intervention, which have the risks of pulmonary edema, myocardial infarction, stroke, and vessel rupture with death and are labor-intensive and expensive.5, 7 Alternative treatments for CVSP offer the hope of improving outcome.

Although CVSP is a multifactorial disease process, the common pathway of vasoconstriction is the continuous elevation of intracellular Ca2+ levels attributable to a combination of influx from extracellular Ca2+ and release from the largest intracellular Ca2+ store, the endoplasmic/sarcoplasmatic reticulum mediated by the ryanodine receptor.8–11 Nimodipine and nicardipine, both L-type-specific Ca2+ channel blockers, have been used for CVSP12, 13 but are only partially effective, possibly because they affect only the influx of extracellular Ca2+ and have no effect on ryanodine receptor-mediated intracellular Ca2+ release. Dantrolene is a known ryanodine receptor inhibitor and is already approved by the U.S. Food and Drug Administration for other indications.14, 15 There is evidence that dantrolene is neuroprotec-
tive.16–20 Furthermore, in an ex vivo rat model, dantrolene has been shown to inhibit cerebral vasoconstriction alone as well as in combination with nimodipine.21 Similarly, a small human study has suggested that dantrolene may attenuate CVSP after SAH.22 Combining L-type-specific Ca2+ channel blockers with a ryanodine receptor blocker may be the key therapeutic target in CVSP.

Up to 30% to 40% of SAH patients may experience hyponatremia for various reasons,23 which may be exacerbated by intravenous (IV) dantrolene given its solution in free water. Further, the combination of nimodipine with dantrolene may theoretically cause hypotension. Therefore, we conducted a prospective, open-label, single-dose ascending safety trial in patients with transcranial Doppler (TCD) as an efficacy biomarker. We proposed that only if this pilot study showed any effect, future larger studies of dantrolene in SAH-related CVSP would be warranted.

Materials and Methods

Study Design

We conducted a prospective, open-label, single-center study using a single infusion of IV dantrolene with 2 dose tiers (http://clinicaltrials.gov; NCT00964548). The study was approved by the Massachusetts General Hospital/Partners Health Care Institutional Review Board. Written informed consent was obtained from all patients or the health care proxies. Using a convenience sample of 10 patients, the first 5 patients received 1.25 mg/kg, whereas the subsequent 5 patients received 2.5 mg/kg. We chose 2.5 mg/kg as the highest dose for 2 reasons: in previous studies of healthy volunteers, maximum grip strength weakness occurred at approximately this dose (2.2 mg/kg)24 and higher doses may pose too high of a risk for respiratory muscle weakness for our mostly nonintubated patient population; furthermore, based on its pharmacological properties, IV dantrolene infusions every 6 hours result in steady dantrolene serum levels.24 Therefore, in concordance with the maximum Food and Drug Administration-approved daily cumulative total dose of 10 mg/kg, a dosing regimen of every 6 hours equates to 2.5 mg/kg IV every 6 hours. To investigate the effect of a lower dose on TCD velocities, we chose half of the high dose (1.25 mg/kg).

Study Protocol

Patients were approached for consent if they met the following inclusion criteria: aneurysmal SAH, as proven by head CT and CTA, nimodipine 60 mg orally every 4 hours. When the daily routine TCD examination suggested evidence of vasospasm, patients were started on hypervolemic hypertensive therapy as standard of care at the Massachusetts General Hospital neuroscience intensive care unit; normal saline infusion at 150 to 200 mL/hr and mean arterial pressure goal >100 mm Hg using phenylephrine with the addition of norepinephrine if needed to achieve the mean arterial pressure goal. Likewise, 250 mL 5% albumin was infused every 6 hours as needed.

Laboratory Measurements

Baseline serum laboratory values were examined before the infusion at 0 minutes (alanine aminotransferase, aspartate transaminase, alkaline phosphatase, arterial blood gas, chemistry, and osmolality). At the end of the study period (135 minutes), all serum laboratory tests were repeated except alanine aminotransferase, aspartate transaminase, and alkaline phosphatase, which were repeated 24 hours after the baseline (t24 hours).

TCD Measurements

At 0 minutes, patients underwent a confirmatory TCD examination (baseline) by 1 of the investigators (S.M. or J.S.) selective to the vessel with elevated velocities and the corresponding contralateral vessel (if applicable) or the vertebral arteries (for basilar artery). TCD throughout the study were repeated by the same examiner and were exclusively selective to remain within the time constraints of the study. A full TCD examination may take ≥30 minutes, blurring...
the time intervals for the TCD aspect of the study, whereas the selective study TCD lasted between 5 and 10 minutes. TCD have been shown to be sensitive in detecting CVSP after SAH when performed by the same experienced examiner.26 TCD were repeated 3 times every 45 minutes: once at 45 minutes during the dantrolene infusion, and twice after the dantrolene infusion at 90 minutes and 135 minutes. Vessels were insonated at predefined depths (in mm from the temporal bone for middle cerebral artery and anterior cerebral artery and occiput for vertebral artery and basilar artery) to standardize the TCD examination and allow calculations of mean change from baseline: the middle cerebral artery was insonated at depths 65, 60, 55, 50, and 45 mm, anterior cerebral artery at 65, 70, and 75 mm, vertebral artery at 60, 65, 70, 75, and 80 mm, and basilar artery at 85, 90, 95, 100, 105, and 110 mm. PSV and MFV were recorded. For each patient, the baseline velocity at the corresponding depth for each vessel in vasospasm was set as zero. For each vessel at each corresponding depth, the change in PSV and MFV from baseline was calculated and averaged for each vessel in vasospasm. TCD were performed with a Spencer PMD 150 TCD machine (Spencer Technologies).

Hemodynamic Measurements
During the infusion period hemodynamic parameters were recorded every 10 minutes (heart rate, blood pressure, central venous pressure, intracranial pressure if applicable, and cerebral perfusion pressure if applicable). From 0 until 135 minutes, the vasopressor dose was left unchanged.

Statistical Analysis
Laboratory data were analyzed using the Wilcoxon sign-rank test. Vital signs and TCD velocities were analyzed using repeated-measures ANOVA. P≤0.05 was considered significant. Post-ANOVA analysis was performed using Bonferroni multiple comparisons test with adjusted P values (P≤0.001). Analyses were performed using SAS 9.2 (SAS Institute) and PRISM 5.03 (GraphPad Software).

Results
Between June 2007 and October 2008, 10 patients received the study drug and completed the study protocol (Figure 2). A total of 16 vessels found to be in TCD vasospasm were included in the analysis. Baseline characteristics are shown in the Table and Supplemental Table 1 (http://stroke.ahajournals.org).

Laboratory Values
No differences were noted in the basic chemistries, osmolality, arterial blood gas, or liver function tests before and after the dantrolene infusion in either dose group (Supplemental Table 2, http://stroke.ahajournals.org).

Hemodynamic Values
In both dose groups, heart rate, mean arterial pressure, intracranial pressure, cerebral perfusion pressure, and central venous pressure remained unchanged during the study period (Supplemental Figure, http://stroke.ahajournals.org). There was a significant decrease in the systolic blood pressure in the low-dose group (maximal, −8 mm Hg; 95% CI, −26 to 10 mm Hg; P=0.027), but not in the high-dose group. Post hoc ANOVA analysis using Bonferroni test revealed that there was not a specific time point during which this decrease in systolic blood pressure occurred. This finding was driven by a single patient. Exclusion of this patient for this particular analysis showed no significant change in the systolic blood pressure in the remaining patients (P=0.28).

TCD Results
We observed a significant decrease from baseline in the mean change of both PSV and MFV in the low dose group (−27 cm/s; 95% CI, −43 to −11 cm/s; P=0.004; and −18 cm/s;
After excluding TCD data from the low blood pressure outlier, the mean change of PSV remained significant (−26 cm/s; 95% CI, −47 to −5 cm/s; \( P = 0.02 \)), with a borderline change in MFV (−16 cm/s; 95% CI, −36 to 4 cm/s; \( P = 0.07 \)). In the high-dose group, there was a trend toward a decrease in PSV (−26 cm/s; 95% CI, −56 to 5 cm/s; \( P = 0.05 \)), but no significant decrease in MFV (−13 cm/s; 95% CI, −34 to 8 cm/s; \( P = 0.14 \); Figure 3). Mean baseline Lindegaard ratios (calculated if the middle cerebral artery was in CVSP) were 3.2±1 for the low-dose group (n=5) and 3.8±0.8 for the high-dose group (n=6). There was no significant change in the Lindegaard ratios in either dose group (at 135 minutes: low-dose mean, 2.8; 95% CI, 1.4–4.2; \( P = 0.72 \); high-dose mean, 3.4; 95% CI, 1.9–5; \( P = 0.24 \)).

**Discussion**

Dantrolene is an inviting new therapeutic agent for CVSP because it has a plausible biological mechanism, is already approved by the Food and Drug Administration, has a well-known and well-tolerated side-effect profile, is neuroprotective,16–20 and may inhibit cerebral vasoconstriction, as shown previously.21,22 Administering IV dantrolene to SAH patients using nimodipine and hypervolemic hypertensive therapy may pose some safety concerns, because 20 mg dantrolene is diluted in 60 mL of sterile water with hypervolemic hypertensive therapy, 5% mannitol and sodium hydroxide, yielding a pH of 9.5.20 Especially in SAH patients, this may lead to hyponatremia, hypotension (particularly in combination with nimodipine), hypoosmolality, and alkalosis. Furthermore, dantrolene may cause liver toxicity.20,27 We conducted this pilot study to explore these safety concerns and the short-term effects of a single dose of dantrolene in this specific patient population.

We did not observe any significant hyponatremia, hypoosmolality, changes in pH, or liver enzyme abnormalities after a single dose. However, such side effects may only become apparent with cumulative doses, and future studies with repeated dantrolene doses should continue to monitor for these.

We observed a decrease in the systolic blood pressure in the low-dose group, but not in the high-dose group. One single outlier in the low-dose group likely explains this finding, because no changes in systolic blood pressure were seen after exclusion of this patient. The etiology of hypotension in this particular instance may be idiosyncratic or a true
systemic effect of nimodipine and dantrolene. No other human studies have linked dantrolene to hypotension, either alone or in combination with calcium channel blockers. In the ischemic swine heart, the combination of amlodipine and dantrolene did not worsen the hypotension and atroventricular nodal conduction delay induced by amlodipine.²⁸ Our study is susceptible to outliers given the small sample size. Future studies warrant close observation of blood pressure during the study period.

Although this study was not powered to study efficacy, the observed changes in TCD velocities are interesting. We detected a significant decrease in the mean change of PSV, with a trend toward decreased MFV in the low-dose group and a trend toward a decrease PSV in the high-dose group. A possible explanation is that the receptor binding and drug kinetics may follow a U-shape curve with less inhibition of vasoconstriction with higher doses. It is unclear how repeated doses affect receptor binding and drug kinetics. We did not evaluate patients for cerebral autoregulation, which may be impaired after SAH. After excluding the single patient with a blood pressure decrease in the low-dose group from the TCD analysis, the mean change in PSV, but not MFV, remained significant, underlining the importance of considering impaired cerebral autoregulation in the analysis of TCD data in SAH.

Limitations
Apparent limitations of our study include the small sample size, which significantly limits statistical analysis and interpretation, the administration of a single dose as opposed to repeated dosing over a longer period of time, the unblinded design, the lack of radiological confirmation of the dynamic changes of CVSP, as well as clinical outcome after dantrolene administration. Our data do not allow us to comment on possible accumulation or end-of-dose or rebound effects because of the short study period (135 minutes). Moreover, we did not study the effect of dantrolene on cerebral blood flow, blood volume, or metabolic changes on brain tissue by means of microdialysis or local tissue oxygenation.

Conclusions
Because of its plausible biological mechanism and its neuroprotective and inhibitory effects on cerebral vasoconstriction, dantrolene might be well-suited for the treatment or prevention of CVSP after SAH. This study shows feasibility and safety of a single infusion of dantrolene, as well as inhibition of TCD vasoconstriction in one dose tier. Future prospective studies using repeated doses of dantrolene over the entire vasospasm period are warranted.

Acknowledgments
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Disclosure
None.

References


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### Supplemental Table 1: Baseline Medications

<table>
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<tr>
<th>Medications</th>
<th>Low dose group (1.25 mg/kg)</th>
<th>High dose group (2.5 mg/kg)</th>
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<tr>
<td>Dilantin</td>
<td>80%</td>
<td>100%</td>
</tr>
<tr>
<td>Phenylephrine (n/mean dose (SD) [mcg/hr])</td>
<td>1 / 230 (21)</td>
<td>3 / 323 (244)</td>
</tr>
<tr>
<td>Norepinephrine (n/mean dose (SD) [mcg/hr])</td>
<td>1 / 3 (0)</td>
<td>1 / 16 (0)</td>
</tr>
<tr>
<td>Propofol (n/mean dose (SD) [mg/hr])</td>
<td>1 / 200 (0)</td>
<td>1 / 200 (0)</td>
</tr>
<tr>
<td>Magnesium infusion (n/mean dose (SD) [mg/hr])</td>
<td>4 / 1.6 (0.2)</td>
<td>3 / 1.3 (0.2)</td>
</tr>
<tr>
<td>3% saline use (n/mean dose (SD) [ml/hr])</td>
<td>1 / 20 (0)</td>
<td>2 / 55 (5)</td>
</tr>
</tbody>
</table>
Supplemental Table 2: Laboratory Values

Values shown are mean (SD). P-values were calculated using the Wilcoxon sign rank test.

<table>
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<tr>
<th>Variable</th>
<th>Before</th>
<th>After</th>
<th>p-value (95% CI of difference)</th>
<th>Before</th>
<th>After</th>
<th>p-value (95% CI of difference)</th>
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<tr>
<td>Na [mmol/dL]</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>n=5, low dose</td>
<td>140 (4.9)</td>
<td>138 (4.4)</td>
<td>0.06 (-5.1; 0.3)</td>
<td>n=5, low dose</td>
<td>7.43 (0.05)</td>
<td>7.43 (0.05)</td>
</tr>
<tr>
<td>n=5, high dose</td>
<td>139 (4.2)</td>
<td>137 (4.3)</td>
<td>0.06 (-3.2; -0.8)</td>
<td>n=5, high dose</td>
<td>7.42 (0.04)</td>
<td>7.44 (0.04)</td>
</tr>
<tr>
<td>Cl [mmol /dL]</td>
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<tr>
<td>n=5, low dose</td>
<td>107 (7)</td>
<td>105 (6)</td>
<td>0.06 (-5; 0.2)</td>
<td>n=5, low dose</td>
<td>38 (4)</td>
<td>38 (6)</td>
</tr>
<tr>
<td>n=5, high dose</td>
<td>104 (4)</td>
<td>101 (5)</td>
<td>0.06 (-5.3; -0.7)</td>
<td>n=5, high dose</td>
<td>42 (8)</td>
<td>40 (8)</td>
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<tr>
<td>Bicarb [mmol /dL]</td>
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<tr>
<td>n=5, low dose</td>
<td>25.7 (3.4)</td>
<td>24.8 (3.5)</td>
<td>0.06 (-1.3; -0.6)</td>
<td>n=5, low dose</td>
<td>119 (48)</td>
<td>96 (41)</td>
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<tr>
<td>n=5, high dose</td>
<td>25.3 (3.6)</td>
<td>26 (3.1)</td>
<td>0.19 (-0.3; 1.6)</td>
<td>n=5, high dose</td>
<td>80 (27)</td>
<td>80 (28)</td>
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<td>BUN [mg/dL]</td>
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<tr>
<td>n=5, low dose</td>
<td>9 (4)</td>
<td>9 (4)</td>
<td>0.5 (-2; 0.8)</td>
<td>n=5, low dose</td>
<td>29 (31)</td>
<td>37 (24)</td>
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<tr>
<td>n=5, high dose</td>
<td>14 (9)</td>
<td>14 (9)</td>
<td>0.5 (-1.1; 0.3)</td>
<td>n=5, high dose</td>
<td>52 (82)</td>
<td>60 (69)</td>
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<tr>
<td>Creatinine [mg/dL]</td>
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<td>n=5, low dose</td>
<td>0.8 (0.36)</td>
<td>0.77 (0.39)</td>
<td>0.5 (-0.1; 0.03)</td>
<td>n=5, low dose</td>
<td>27 (20)</td>
<td>35 (14)</td>
</tr>
<tr>
<td>n=5, high dose</td>
<td>0.84 (0.16)</td>
<td>0.9 (0.16)</td>
<td>0.13 (-0.03; 0.2)</td>
<td>n=5, high dose</td>
<td>35 (23)</td>
<td>44 (20)</td>
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<tr>
<td>Glucose [mg/dL]</td>
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<td></td>
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<tr>
<td>n=5, low dose</td>
<td>141 (37)</td>
<td>133 (24)</td>
<td>0.31 (-32.9; 17.3)</td>
<td>n=5, low dose</td>
<td>71 (16)</td>
<td>80 (13)</td>
</tr>
<tr>
<td>n=5, high dose</td>
<td>133 (24)</td>
<td>135 (39)</td>
<td>1 (-73.8; 79.4)</td>
<td>n=5, high dose</td>
<td>95 (37)</td>
<td>108 (35)</td>
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<tr>
<td>Osmolality [mOsm/kg]</td>
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<tr>
<td>n=5, low dose</td>
<td>293 (9)</td>
<td>291 (6)</td>
<td>0.56 (-6.9; 3.7)</td>
<td>n=5, low dose</td>
<td>293 (13)</td>
<td>294 (8)</td>
</tr>
<tr>
<td>n=5, high dose</td>
<td>293 (13)</td>
<td>291 (6)</td>
<td>0.56 (-6.9; 3.7)</td>
<td>n=5, high dose</td>
<td>293 (13)</td>
<td>294 (8)</td>
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</tbody>
</table>
Supplemental Figure: Hemodynamic measurements

In the low dose group (panel A), a significant change (*) was seen in the systolic blood pressure (SBP). Post-hoc Bonferroni test with adjusted p-value (p ≤ 0.001) for multiple comparisons revealed no significant difference in SBP between specific time points. No other changes in any of the measured hemodynamic parameters were seen in the low dose (panel A) or high dose group (panel B). Shown are mean values with standard deviation.

Abbreviations: HR, heart rate; MAP, mean arterial pressure; ICP, intracranial pressure; CPP, cerebral perfusion pressure; CVP, central venous pressure.