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Prevention of delayed cerebral vasospasm by continuous intrathecal infusion of glyceroltrinitrate and nimodipine in the rabbit model in vivo

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Abstract Objective: Intrathecal bolus administration of nitric oxide donors and calcium channel antagonists has been proposed to reduce cerebral vasospasm (CVS) in animal subarachnoid hemorrhage (SAH) models. Intrathecal continuous administration of these substances for CVS prevention has not been extensively evaluated. This study compared the efficacy of continuous intrathecal infusions of the NO donor glyceroltrinitrate and nimodipine in preventing delayed CVS associated with SAH in an animal model in vivo. **Methods:** New Zealand White rabbits were randomly assigned to six groups: no SAH/NaCl, no SAH/NO, no SAH/nimodipine, SAH/NaCl, SAH/NO, or SAH/nimodipine. Glyceroltrinitrate (GTN) at 0.5 µg/µl (0.5 µl/h) or nimodipine at 0.2 µg/µl (10 µl/h) or NaCl was continuously infused into the cisterna magna via an Alzet osmotic pump from day 0 to day 5 after injection of 1.0 ml autologous blood. The magnitude of spasm in the basilar artery was determined by comparison of pre- and posttreatment angiography

and was calculated as proportional change in intraluminal diameter based on automatic measurements. **Results:** A total of 55 experiments and 110 angiograms were performed. SAH was associated with vasoconstriction of the basilar artery (SAH/NaCl group 19.85 ± 2.94%). Continuous intrathecal injection of GTN and nimodipine prevented SAH-induced CVS. There was significant prevention of CVS in animals treated with GTN (SAH/NO group 5.93 ± 5.2%, n = 11) and nimodipine (SAH/nimodipine group: 0.55 ± 2.66%, n = 9). There was no significant difference between the treatment groups and controls in prevention of CVS. **Conclusions:** This study demonstrates that prophylactic continuous intrathecal administration of either GTN or nimodipine equally prevents SAH-associated CVS in an animal model.

Keywords Subarachnoid hemorrhage · Vasospasm · Animal models · Nitric oxide · Nimodipine

Introduction

Symptomatic delayed cerebral vasospasm (DCV) develops in approx. 30% of patients after aneurysmal subarachnoid hemorrhage (SAH) and remains one of the major causes of serious morbidity and death [1, 2]. Brain ischemic events secondary to cerebral vasospasm (CVS) classically

occur 4–12 days after initial bleeding [3]. The exact cause of CVS is unknown despite awareness of the entity for almost half a century. Although the treatment protocol of hypervolemia, hemodilution, and hypertension (“triple-H therapy”) in combination with intravenous nimodipine administration has had a substantial beneficial effect in CVS treatment [4], in some patients the protocol is not tolerated

or is ineffective [5–7]. Despite our increased knowledge of the molecular basis of DCV following SAH no current treatment modalities offer prophylactic prevention. Preliminary case series in humans [8–11] and experimental studies in a variety of animal models [12–17] suggest that intrathecal administration of an NO donor would be an effective treatment for reversing CVS. Controlled-release polymers such as diethyl-amine-triamine/NO and ethylene-vinyl acetate copolymer implanted in the subarachnoid space close to the experimental bleeding site serve as NO donors in most recent animal models with intrathecal administration of the drugs [12–18]. Furthermore, sodium nitroprusside (SNP) and glyceroltrinitrate (GTN) have served as NO donor to prevent CVS after SAH [8–11, 19, 20].

Nimodipine, the most widely administered calcium channel blocker, is a dihydropyridine calcium channel antagonist that has been shown to decrease the incidence of overall cerebral infarction after SAH by 34% and the incidence of poor outcomes by 40% [21]. Whereas intravenous or oral administration of nimodipine does not appear to decrease the incidence of angiographic CVS [22, 23], intraoperative bathing of vessels in nimodipine solution and intrathecal bolus administration has demonstrated reversal of spasm and dilatation of nonspastic vessels, without changes in mean arterial blood pressure [24–29]. The present study compared the efficacy of continuous intrathecal infusions of the NO donor glyceroltrinitrate and nimodipine in preventing CVS in the rabbit SAH model in vivo.

Materials and methods

The protocol of this study was reviewed and approved by the Swiss Institutional Animal Care and Use Committee

and meets the Swiss guidelines for laboratory animal use (Department of Veterinary Service, Berne, Switzerland, approval no. 125/05).

Study design

A total of 55 adult New Zealand White rabbits of either sex weighing 2.6–3.6 kg were randomly assigned to six experimental groups. The groups did not differ significantly in body weight, PaCO₂, mean arterial blood pressure, or heart rate at baseline (day 0) or at follow-up (day 5; Table 1).

Anesthesia, monitoring, and killing

Induction of general anesthesia was performed by intramuscular administration of ketamine (30 mg/kg; Ketalar, 50 mg/ml, Pfizer, Zurich, Switzerland) and xylazine (6 mg/kg; Xylapan 20 mg/ml, Vetoquinol, Bern, Switzerland). During surgery pain was evaluated periodically by toe-pinch. Postoperative pain relief was managed by subcutaneous administration of buprenorphine (0.1–0.2 mg/kg) for up to 36 h after SAH. Heart rate and blood pressure were monitored throughout the digital subtraction angiography (DSA) on day 0 and day 5 after experimental SAH. Intravascular pressures and heart rate were measured with a Camino multiparameter monitor (Integra, Plainsboro, NJ, USA). On the same days arterial blood gas status was analyzed to monitor oxygenation parameters from a collected arterial blood sample (ABL 725, Radiometer, Copenhagen, Denmark). The animals underwent daily clinical observation. The animals were killed on day 5 post-SAH induction under the same anesthetic as the angiography by intra-arterial bolus injection of sodium thiopental (40 mg/kg) (Pentothal, Ospedalia,

Table 1 Measurements of physiological parameters (*PaCO*₂, arterial carbon dioxide pressure; *PaO*₂, arterial oxide pressure; *MABP*, mean arterial blood pressure; *HR*, heart rate)

	Body weight (kg)	PaCO ₂ (mmHg)	PaO ₂ (mmHg)	MABP (mmHg)	HR (per min)
no SAH/NaCl					
Day 0	3.6 ± 0.1	51.70 ± 0.95	50.52 ± 8.6	65.5 ± 4.04	162 ± 5
Day 5	3.6 ± 0.1	43.60 ± 3.30	57.20 ± 1.3	56.20 ± 6.64	174 ± 7
no SAH/NO					
Day 0	3.7 ± 0.2	44.00 ± 1.97	54.72 ± 3.7	64.33 ± 2.89	157 ± 4
Day 5	3.7 ± 0.2	45.75 ± 7.34	57.31 ± 4.1	55.33 ± 4.96	155 ± 8
no SAH/nimodipine					
Day 0	2.7 ± 0.1	49.43 ± 1.82	63.24 ± 4.2	77.83 ± 3.43	178 ± 10
Day 5	2.7 ± 0.1	50.23 ± 1.77	61.04 ± 4.9	70.50 ± 2.62	181 ± 6
SAH/NaCl					
Day 0	3.8 ± 0.2	45.37 ± 1.63	67.27 ± 5.2	72.50 ± 3.95	163 ± 4
Day 5	3.8 ± 0.2	41.36 ± 3.68	63.07 ± 4.1	59.83 ± 2.68	171 ± 7
SAH/NO					
Day 0	3.8 ± 0.1	43.52 ± 2.16	64.80 ± 3.2	67.33 ± 3.40	179 ± 5
Day 5	3.8 ± 0.1	44.03 ± 2.57	62.98 ± 8.5	65.00 ± 4.95	163 ± 7
SAH/nimodipine					
Day 0	3.1 ± 0.2	48.68 ± 1.43	67.05 ± 3.4	75.83 ± 2.52	191 ± 9
Day 5	3.1 ± 0.2	48.30 ± 1.68	74.07 ± 6.5	71.17 ± 2.09	178 ± 7

Hünenberg, Switzerland). Postmortem examination of operative scars, pump, and tube position was performed to evaluate signs of infection and to affirm correct silicone catheter connection to the pump and unchanged catheter location within the subarachnoid space of the atlanto-occipital cistern.

Angiography

DSA was performed under general anesthesia on day 0 prior to SAH and on day 5 post-SAH. The rabbit's left (day 0) or right (day 5) subclavian artery was microsurgically exposed and cannulated using a 0.5 mm plastic tube. The catheter was introduced retrogradely and fixed distally to the origin of the vertebral artery. Subsequently the DSA was performed by intra-arterial bolus injection of nonionic iopamidol (0.6 ml/kg; Abott Laboratories, North Chicago, IL, USA) as contrast agent. Images of the vertebrobasilar system were obtained using a rapid sequential angiographic technique with the small focal spot at 66 kV, 125 mA (DFP 2000A, Toshiba, Japan). Measurement of each vessel was performed three times in a blinded fashion by using the automatic measurement tool of the ImagePro Discovery analysis software (MediaCybernetics, Silver Spring, MD, USA), and mean values were determined.

Induction of experimental SAH

Following baseline DSA on day 0, the atlanto-occipital membrane was exposed, and a 25-gauge needle was inserted into the cerebromedullar cistern. After relief of 1.0 ml CSF an equal amount of unheparinized autologous arterial blood was injected into the cistern under microscopic view. The rabbits were kept positioned at a 65° angle head-down for 10 min to allow blood dissemination throughout the subarachnoid space (Fig. 1).

Miniosmotic pump implantation

Depending on the study group, a miniosmotic pump (0.5 μ l/h, Alzet Osmotic Pumps, Durect, Cupertino, CA, USA) containing 0.5 mg/ml GTN solution (Perlinganit, Schwarz Pharma, Münchenstein, Switzerland) or a miniosmotic pump (10 μ l/h, Alzet) containing 0.2 mg/ml nimodipine solution (Nimotop, Bayer HealthCare, Cologne, Germany) was implanted subcutaneously. Dose-response curves of intrathecal GTN and nimodipine are not available. The dose of GTN was derived from studies in humans in which up to 8 mg/24 h was used for prophylaxis against vasospasm [8, 9]. The pH value of the GTN and nimodipine solution was 7.35 and 7.4, respectively. The dose of nimodipine was calculated from studies in

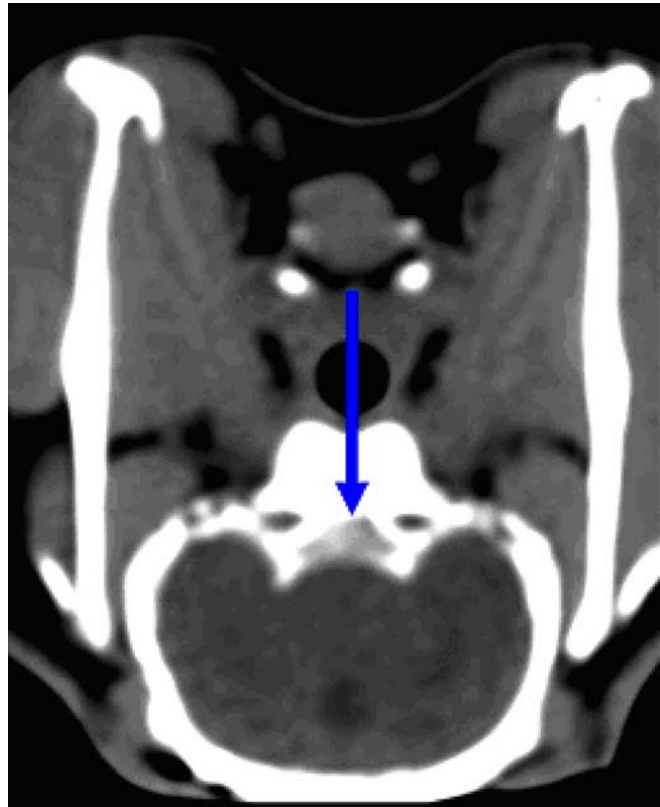


Fig. 1 Axial computer tomography demonstrates the successful dissemination of blood after cisterna magna injection. *Arrow*, Hyperdense prepontine blood collection

canine models [26, 27]. The rabbit's CSF volume was estimated to be 70–80 times less than that in humans and 5–10 times less than that in dogs [30, 31]. Pumps, associated tubing, and drugs were protected from light at all times. A silicone catheter (STH-C040, Connectors Verbindungstechnik, Tagelswangen, Switzerland) was connected with the pump and inserted at least 1 mm through the atlanto-occipital membrane into the subarachnoid space. The tube insertion site was sealed by a muscle patch and fixed by several stitches. The midline spinal muscle incision was closed in two layers. Finally, the wounds were superficially irrigated with neomycin sulfate (Research Organics, Cleveland, OH, USA) for infection prophylaxis. All surgical procedures were performed under sterile conditions.

Statistical methods

Values were expressed as means of each group \pm SEM (n = number of animals). Statistical significance between two means was determined by Student's unpaired *t* test and significance between multiple means was determined by analysis of variance.

Results

Mortality, morbidity, and neurological status

Two animals died immediately after injection of autologous blood into the atlanto-occipital cistern as a result of respiratory arrest. All other animals were in bland neurological condition after the angiographic and surgical procedures and did not develop delayed neurological deficits, with the exception of one animal in the SAH/NaCl control group, which exhibited transient paresis of the hind legs on day 2 after experimental SAH. Clinical daily follow-up of the animals was uneventful up to day 5 when they were killed. Gross pathological evaluation revealed no signs of infection. All of the implanted pump devices were placed in accurate position 1–2 mm underneath the atlanto-occipital membrane.

Angiographic measurements

A total of 110 angiographic vessel diameters of the rabbit basilar artery were investigated in the six groups. Angiographic measurements and diameter changes in percentages are given in Table 2 and Fig. 2, respectively. The baseline vessels' diameter on day 0 did not differ significantly among the six experimental groups. The data show that SAH and plain saline infusion were associated with vasoconstriction of the basilar artery (SAH/NaCl group $19.85 \pm 2.94\%$; no SAH/NaCl group $14.92 \pm 4.49\%$; Table 2). The continuous intrathecal infusion of both nimodipine and GTN significantly reduced SAH-associated vasoconstriction (SAH/nimodipine $0.55 \pm 2.66\%$, $n = 9$, vs. SAH/NaCl $19.85 \pm 2.94\%$, $n = 8$, $p < 0.005$; SAH/NO $5.93 \pm 5.2\%$, $n = 11$, vs. SAH/NaCl: $19.85 \pm 2.94\%$, $n = 8$, $p < 0.05$). The comparison of the effects of nimodipine and GTN on diameter changes after SAH showed no statistical difference.

Discussion

The results of this study demonstrate nearly complete prevention of delayed CVS of the rabbit basilar artery in response to continuous intrathecal infusion of GTN or

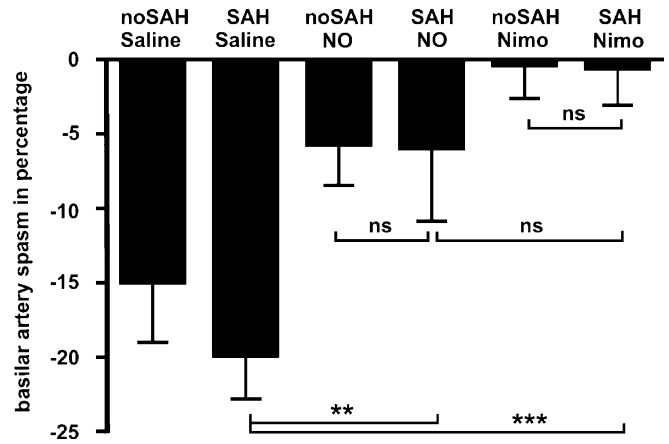


Fig. 2 Bar graph shows the results of the effect of continuous intracisternal administration of nitric oxide donor (nitroglycerin) and nimodipine on cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits in vivo. The SAH/NO donor group ($n = 11$) and the SAH/nimodipine group ($n = 9$) both demonstrate significant prevention (** $p = 0.0031$, *** $p = 0.0002$) of the basilar artery spasm compared to the control SAH/Saline group ($n = 8$) 5 days postexperimental subarachnoid hemorrhage. There was no significant ($p = 0.3741$) difference in the efficacy of spasm reduction between the SAH/NO donor group and the SAH/nimodipine group. There was also no significant difference between the no SAH/NO group ($n = 9$) and the SAH/NO group ($p = 0.9622$) or between the no SAH/nimodipine ($n = 7$) group and the SAH/nimodipine group ($p = 0.9507$). *Nimo*, Nimodipine

nimodipine and therefore confirms previous promising results with intrathecal use of these two compounds [12–17, 24–29, 32]. In the presented experimental setting they showed no significant difference in their efficacy.

NO is the smallest biologically active molecule known. As a highly diffusible gas it mediates an extraordinary range of physiological processes [33, 34]. The role of NO in the context of SAH began when the identity of the hypothetical endothelium-derived relaxing factor and NO was recognized [35–37]. Ongoing research revealed further extensive roles of NO in neurotransmission, inflammation, and vascular autoregulation [38]. In particular NO appears to decrease vascular inflammation (one of the key pathways in delayed CVS) [39, 40], serves as a high potent vasodilating agent [41], and regulates the

Table 2 Summary of the effect of continuous intrathecal nitroglycerin and nimodipine infusion on basilar artery caliber (μm) in rabbits with experimental SAH. Values are expressed as the mean \pm standard error of mean

	<i>n</i>	Diameter (μm) Day 0	Day 5	Diameter reduction (%)
no SAH/NaCl	11	669.51 \pm 2.97	593.59 \pm 3.03	14.92 \pm 4.49
no SAH/NO	9	676.03 \pm 1.27	625.18 \pm 1.14	5.67 \pm 2.92
no SAH/nimodipine	7	730.04 \pm 2.60	733.88 \pm 5.90	0.32 \pm 2.30
SAH/NaCl	8	688.71 \pm 1.81	542.60 \pm 1.70	19.85 \pm 2.94
SAH/NO	11	648.21 \pm 1.38	637.26 \pm 2.26	5.93 \pm 5.20**
SAH/nimodipine	9	726.58 \pm 3.85	713.68 \pm 3.18	0.55 \pm 2.66*

* $p < 0.001$, ** $p < 0.05$ vs. control group SAH/NaCl

cerebral vascular tone as antagonist of the most potent vasoconstrictor ET-1 [42]. Due to the short half-life, side effects, and potential toxicity of NO in clinical use, investigators in the past have focused on treating experimental CVS by using intrathecal and periadventitial soluble administration or polymer placements of NO and NOD (see Table 1, see ESM) [12–17]. Most of the performed animal studies used diethylenetriamine nitric oxide donor from a controlled release polymer to prevent CVS after experimental SAH [13, 15–17]. Thomas et al. [8] and Kumar et al. [11] published preliminary results of effective treatment of intrathecal bolus administration of SNP in patients with established delayed CVS. Raabe et al. [10] studied the effect of SNP in patients with severe, medically refractory vasospasm. They suggested that SNP is more effective when initiated early and administered continuously.

The highly lipid-soluble calcium antagonist nimodipine has been advocated in the treatment of cerebral vasospasm because it inhibits a fundamental mechanism involved in vasoconstriction of cerebral arteries, calcium influx. The influx of extracellular calcium ions results from the opening of L type calcium channels (target of calcium antagonists) and additional channels such as store operated-channels [43]. Lack of prevention of CVS and systemic side effects with oral or intravenous administration led to experiments with topical application. Voldby et al. [26] studied the effect of intrathecal bolus administration of calcium antagonist nimodipine after experimental SAH in dogs. Nimodipine always relieved spasm and often the relaxation surpassed the resting vessel diameter, whereas in a control group the injection of placebo did not relax the spastic arteries [26]. Goia et al. [27] and Zabramski et al. [32] found beneficial effect on intrathecal nimodipine as opposed to systemic administration in reversing angiographic vasospasm in the two-hemorrhage and multihemorrhage canine models. Based on these promising data derived from experimental studies on topical nimodipine, first clinical trials have been carried out using perivascular administration of the drug [24, 25, 28]. Despite promising results from these trials and previous experimental studies (Table 2, ESM) [24–29, 32], intrathecal administration of nimodipine as a potential avenue in CVS prevention remained controversial and fall into oblivion [29].

Not only the systemic side effects of current treatment modalities but also the trend towards endovascular treatment of ruptured aneurysms has influenced research into alternatives for treating CVS after SAH [44, 45]. During the procedure of craniotomy and aneurysm clipping the vessels surrounding the bleeding site are accessible for topical drug application or deposition of a polymer or placement of a tube for repeated drug administration. Methods which require surgical access to the region of interest are less likely to be used in a patient treated with endovascular coiling. Other approaches are likely to generate more

interest, such as administration through lumbar puncture and advancement of a microcatheter and microguidewire to the ventral cisterna magna under fluoroscopic visualization or administration via conventional external ventricular drain.

In bleeding sites, independently of intrathecal drug application, the ability of the drug to reach the target may be impaired by clotting or abnormal cerebrospinal fluid circulation. Thomas et al. [8, 9] argue that the drug (in their experiment SNP) may penetrate the brain parenchyma by way of the ventricle's ependyma. In this hypothesis the drug becomes independent of ventricular CSF circulation but still does not bypass the problem of clot penetration. It remains unclear whether a high drug concentration in close vicinity to the clot is necessary.

In the presented model intrathecal administration is carried out at the same location where the experimental SAH occurred. Nearly all experiments performed are based on this principle of drug administration at the bleeding site. This selectivity is barely conceivable in a clinical setting. Therefore the results from the current study should be evaluated in a model in which the SAH bleeding site is independent of the intrathecal drug administration.

The remarkable vasospasms in the control group (no SAH/NaCl) may have occurred due to aseptic inflammatory response to mechanical disruption [46]. In the beginning of experimental CVS research mechanical stimulation was used to induce arterial narrowing [46, 47]. Other authors with a similar setting of continuous intracisternal drug infusion to attenuate SAH induced CVS do not report a control group of saline infusion and therefore the possibility to compare our findings lacks [48]. However, the results indicate that the placement of drug administration devices in the vicinity of the examined vessel could be unfavorable. Independent location of drug administration from the bleeding site would avoid any local interaction and drug penetration to the affected vessel could be determined. Eligible would be the use of a ventriculosubarachnoid cannula, with the possibility of continuous drug administration and simultaneous measurement of intracranial pressure through the same cranial screw device [49]. In this set-up possible side effects of additional fluid derived from drug administration can be monitored by the ICP parameter.

The results show no statistically significant difference between no SAH/treatment and SAH/treatment groups, which indicates that both nimodipine and GTN are potent in preventing delayed cerebral vasospasm associated with either experimental SAH or pump implantation. In the current understanding of delayed cerebral vasospasm nimodipine and NO donor GTN are acting at the bottom of the pathophysiological cascade and may prevent vasospasm on an unspecific basis, irrespective of the initially underlying cause (chemical stimulation by subarachnoid blood or mechanical stimulation by pump implantation).

The findings of this study warrant further investigations into the dose-dependency effects of continuous intrathecal infusion NO donors and nimodipine in a modified rabbit model with intraventricular drug delivery. Based on current clinical treatment modalities after SAH, an experimental setting with an intrathecal administration site that differs from the bleeding site is needed to confirm these results as

a potential preventive treatment option in SAH-associated CVS.

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