Effects of iodinated contrast media in a novel model for cerebral vasospasm

Efeitos do meio de contraste iodado em um novo modelo de vasoespasmo cerebral

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ABSTRACT
Objective: We developed an in vitro model for vasospasm post subarachnoid hemorrhage that was suitable for investigating brain vessel autoregulation. We further investigated the effects of iodinated contrast medium on the vascular tone and the myogenic response of spastic cerebral vessels.

Method: We isolated and perfused the superior cerebellar arteries of rats. The vessels were pressurized and studied under isobaric conditions. Coagulated blood was used to simulate subarachnoid hemorrhage. The contrast medium iodixanol was applied intraluminally.

Results: Vessels exposed to blood developed significantly stronger myogenic tone (65.7 ± 2.0% vs 77.1 ± 1.2% of the maximum diameter, for the blood and the control group, respectively) and significantly decreased myogenic response, compared with the control groups. The contrast medium did not worsen the myogenic tone or the myogenic response in any group.

Conclusion: Our results show that deranged myogenic response may contribute to cerebral blood flow disturbances subsequent to subarachnoid hemorrhage. The contrast medium did not have any negative influence on vessel tone or myogenic response in this experimental setting.

Keywords: brain ischemia, cerebral angiography, contrast media, hemodynamics, intracranial aneurysm, intracranial vasospasm, subarachnoid hemorrhage.

RESUMO
Objetivo: Desenvolvemos um modelo in vitro para vasoespasmo subsequente à hemorragia subaracnóide que foi adequado para investigar a autorregularão dos vasos cerebrais. Em seguida investigamos os efeitos do meio de contraste iodado no tônus vascular e na resposta miogênica dos vasos cerebrais espásticos. Método: Isolamos e perfundimos as artérias cerebelares superiores de ratos. Os vasos foram pressurizados e estudados em condições isobáricas. Sangue coagulado foi utilizado para simular hemorragia subaracnóide. O meio de contraste iodixanol foi aplicado intraluminarmente. Resultados: Os vasos expostos ao sangue desenvolveram aumento significativo do tônus miogênico (65.7 ± 2.0% vs 77.1 ± 1.2% do maior diâmetro, para o grupo de sangue e o grupo controle, respectivamente) com resposta miogênica significativamente menor do que aquela dos controles. O meio de contraste iodado não piorou o tônus miogênico ou a resposta miogênica em nenhum dos grupos. Conclusão: Nossos resultados mostram que uma resposta miogênica pode contribuir para as alterações de fluxo sanguíneo cerebral subsequentes à hemorragia subaracnóide. O meio de contraste iodado não teve nenhuma influência negativa no tônus vascular ou na resposta miogênica neste modelo experimental.

Palavras-chave: isquemia cerebral, angiografia cerebral, meio de contraste, hemodinâmica, aneurisma intracraniano, vasoespasmo intracraniano, hemorragia subaracnóide.

Cerebral delayed vasospasm is a severe complication following spontaneous subarachnoid hemorrhage (SAH). Vasospasm is an important cause of death and contributes 10-12% to the overall mortality after SAH, which reaches approximately 50% within the first month1,2,3,4.

There is nowadays no specific therapy or prophylaxis for vasospasm2,3,5,6. The low success of current treatment strategies for vasospasm may be due to insufficient knowledge about the pathophysiology of vasospasm, despite a large number of in vitro and in vivo studies done to discover spastic mechanisms and to find an adequate treatment for cerebral vasospasm6,7.

The majority of studies on the mechanism of vasospasm were performed using in vivo animal models6,7. While in vivo
studies successfully reproduce the clinical picture of vasospasm, there is high individual variability in their results, what limits their application for studying effects of vasospasm on mechanisms of cerebral blood flow regulation. The use of in vitro models, where conditions like intraluminal pressure, oxygen tension, and milieu surrounding the vessels are constant, might reduce these disadvantages. Unfortunately, current in vitro models of vasospasm that use whole blood to simulate SAH are technically unsuitable for investigation of the autoregulation of cerebral vessels, mostly because presence of blood prevents visualization of a pressurized vessel and diameter measurements. We developed a novel, highly reproducible and technically uncomplicated model of vasospasm in vitro using a perfusion myograph and videomicroscopy to investigate the myogenic response of cerebral vessels – one of the main mechanisms of cerebral blood flow autoregulation.

Most diagnostic and therapeutic procedures needed for the treatment of SAH patients require the use of iodinated contrast media (CM). It has been shown that CM have adverse effects on vessels of the kidney and other vascular beds. Further, CM influence some components of cerebral blood flow regulation in healthy subjects. We thus tested the hypothesis that CM negatively influence the autoregulation of cerebral blood flow after SAH.

METHOD

All animal handling and experiments were performed in accordance to the guidelines of the Office for Health and Social Matters of Berlin (Berlin, Germany).

Isolation and preparation of cerebral vessels

Adult male Sprague Dawley rats (Charles River, Germany), 150-200 grams (7-8 weeks old), were anaesthetized using isoflurane (Abbott, Baar, Switzerland) and decapitated. Brains were excised and placed in ice-cold preparation physiological salt solution. Segments of superior cerebellar arteries without branches in a diameter of about 250 µm were isolated under magnification using sharpened forceps and microscopic scissors, and mounted on glass cannulas within the myograph’s experimental chamber. As usual in experiments investigating myogenic response, there was no flow inside the vessel.

Altogether 73 rats were included in the study. 50 successful experiments were performed. Excluding criteria were: intraluminal flow due to lacks or vessel branches and insufficient development of spontaneous myogenic tone (< 20%). Experimental groups and number of experiments are shown in Table.

Experimental conditions

Intraluminal

The intraluminal solution consisted of experimental physiological salt solution (PSS) (146 mmol/l NaCl, 4.5 mmol/l KCl, 1.2 mmol/l NaH2PO4, 1.0 mmol/l MgSO4, 1.6 mmol/l CaCl2, 5.5 mmol/l glucose, 0.025 mmol/l EDTA, 5.0 mmol/l HEPES, pH 7.4 by temperature 37.0°C). The groups which received CM had an end concentration of 23 mg iodine/ml (1.8*10⁻⁴ mol/l) (iodixanol, GE Healthcare, Munich, Germany) in the intraluminal solution. The CM concentration is the same shown to cause constriction of renal vasa recta and afferent arterioles, and is within the range possibly reached during intravascular procedures in humans.

Extraluminal inside the chamber

The experimental chamber was filled with PSS and warmed up to 37.0°C. The vessels were exposed to an initial intraluminal pressure of 80 mmHg.

Model of SAH: Fresh blood (2 ml) was collected through laparotomy and sectioning of the renal artery, and deposited into the experimental chamber (0.5 ml in each corner) of a perfusion myograph (model 110P, DMT, Aarhus Denmark). The blood was left to coagulate for 40 minutes by room temperature, during which suitable brain vessels were isolated and prepared for perfusion. The experimental chamber was then filled with the PSS, and the arteries were mounted onto the glass cannulas. There was no contact between the blood clot and the arteries.

Measurement of the vessel diameter and quantification of the myogenic response

The procedure for mounting of the artery, intraluminal pressure manipulation as well as measurement of vessel diameters was in accordance to the principles of investigating of myogenic responses in pressurized arteries. Arterial diameter was recorded in a continuous manner.

Table. The experimental groups according to experimental chamber and intraluminal content of the vessel.

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Experimental chamber content</th>
<th>Intraluminal content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (n = 22)</td>
<td>PSS</td>
</tr>
<tr>
<td>2</td>
<td>Blood (n = 13)</td>
<td>PSS + blood clot</td>
</tr>
<tr>
<td>3</td>
<td>CM (n = 9)</td>
<td>PSS</td>
</tr>
<tr>
<td>4</td>
<td>CM + blood (n = 6)</td>
<td>PSS + blood clot</td>
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CM: Contrast medium; PSS: Physiological salt solution.
over time using an acquisition system consisting of a video camera assembled on an inverted microscope and connected to a software for automatic vessel diameter measurement (IonWizard 6.1, IonOptics, Milton, MA, USA) (Figure 1). Right after mounting of superior cerebellar arteries on glass pipets, continuous diameter measurement was started, and all vessels underwent a period of stabilization of 1 hour during which the development of myogenic tone was monitored. Only arteries which developed typical spontaneous myogenic tone – for cerebral vessels more than 20% of constriction – were included into the study. The value of the myogenic tone was expressed as the percentage of the vessel diameter at the end of stabilization time in relation to the diameter in the maximal dilated state. After recording of baseline diameter measurements, the intraluminal pressure was changed in a controlled manner in 4 steps (each of 5 minutes): 1 step – from 80 to 40 mmHg; 2 step – from 40 to 80 mmHg; 3 step – from 80 to 120 mmHg; and 4 step – from 120 to 80 mmHg. The myogenic response was quantified in micrometers (μm) as the difference between the diameter immediately preceding the pressure step and the diameter measured 5 minutes following the pressure step, when the vessel diameter is stable. All diameter values were measured at the external border of the vessel wall. Figure 2 shows a representative tracing of a typical experiment, where the protocol steps are graphically depicted.

STATISTICAL ANALYSIS

Only one artery was used from each rat for each experiment. Calculation of power was previously performed to determine the optimal sample size. The IBM SPSS Statistics 22 software was used for statistical comparisons. Statistical significance was considered for p-value smaller than 0.05.

Data from spontaneous myogenic tone were reported in the text and in Figure 3 as average and standard error of the means (SEM). Two-way/repeated measurements ANOVA was used to compare groups for spontaneous myogenic tone. Bonferroni method was used as correction for multiple comparisons. Where ANOVA pointed to differences in myogenic tone between groups, Tukey HSD test was used as a post hoc test to compare groups pairwise.

Data from the myogenic response were reported in the text as medians (with 25th and 75th percentiles), and in the Figure 4 as box-plots. The myogenic response steps among all four groups were first compared with the Kruskal-Wallis test. Where ANOVA pointed to differences in myogenic tone between groups, Tukey HSD test was used as a post hoc test to compare groups pairwise.
Vessels exposed to CM and blood clot together had a final diameter compared to control groups: with blood developed a more pronounced myogenic tone ($p = 0.000002$), while CM did not ($p = 0.423$). Vessels treated with blood and CM together showed a significant decrease of both the dilatory and the constrictor myogenic response (80-40 mmHg: 9.1 μm, 6.2 μm, 10.8 μm, $p = 0.00004$; 40-80 mmHg: 1.7 μm, 0.5 μm, 3 μm, $p = 0.0002$; 80-120 mmHg: 10.9 μm, 7 μm, 18 μm, $p = 0.0006$; 120-80 mmHg: -6.4 μm, -23.1 μm, -2.5 μm, $p = 0.008$) in comparison to the control group, respectively (Figure 4).

Vessels treated with blood alone showed a significant decrease of the first and second steps of the myogenic response (80-40 mmHg: -3.3 μm, -7.9 μm, -1.9 μm, $p = 0.00004$; 40-80 mmHg: -9.3 μm, -13.1 μm, -7.1 μm, $p = 0.024$; 80-120 mmHg: -0.1 μm, -4.1 μm, 4 μm, $p = 0.84$; 120-80 mmHg: 1.7 μm, -2.8 μm, 4.3 μm, $p = 0.89$) compared to the control group, respectively (Figure 4).

The myogenic response differed between vessels treated with blood and CM together compared to blood alone only at the third step of pressure (80-40 mmHg: $p = 0.55$; 40-80 mmHg: $p = 0.072$; 80-120 mmHg: $p = 0.0032$; 120-80 mmHg: $p = 0.099$; Figure 4).

**DISCUSSION**

The focus of our study was to assess the effect of a contemporary, widely used CM on the myogenic response of spastic cerebral vessels. A novel in vitro model was developed to simulate vasospasm post SAH. It consisted of deploying a controlled amount of blood clot in the experimental chamber without mechanical interaction with the vessel.

The mechanisms of vasospasm post SAH have been investigated using different models. There are in vivo and in vitro approaches. The most common in vivo techniques are: injection of blood into the brain cisternae and vessel avulsion$^{22,23}$. Some investigators placed blood clots into the brain$^{18}$. In vitro models include application of whole blood$^{89}$ or of vasoactive substances which are hypothetically involved in the development of vasospasm$^{18}$ into the organ bath solution.

In our experience models of SAH using whole blood$^{89}$ were not suitable for the investigation of spontaneous

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**RESULTS**

**Spontaneous myogenic tone**

Two-way/repeated measurements ANOVA followed by Tukey HSD test showed that exposure to blood significantly influenced the development of myogenic tone over time ($p = 0.000002$), while CM did not ($p = 0.423$). Vessels treated with blood developed a more pronounced myogenic tone compared to control groups:

- Vessels treated with blood clot alone had a final diameter of $65.7 + 2.0\%$ of maximal diameter after stabilization time, compared to $77.1 + 1.2\%$ of the control group (average ± SEM, $p = 0.00001$);
- Vessels exposed to CM and blood clot together had a final diameter of $62.1 + 2.1\%$ of maximal diameter, compared to $77.2 + 1.7\%$ of the CM group (average ± SEM, $p = 0.00024$).

**Myogenic response**

There were statistically significant differences between the experimental groups in all four steps of the myogenic response measurement (Kruskal-Wallis test, $p < 0.05$). Figure 4 shows the magnitude of variation in vessel diameter in response to controlled changes in intraluminal pressure, as well as which experimental groups differed from another in each step of the protocol (Mann-Whitney-test). Exposition to blood alone impaired the myogenic response in all steps of pressure. CM alone did not influence the myogenic response in comparison to the control group. In the groups exposed to blood, CM influenced the myogenic response only in the third step of the protocol (80 to 120 mmHg) when compared to blood alone.

In the control group, the change of intraluminal pressure from 80 to 40 mmHg dilated vessels by 11.9 μm (25th and 75th percentiles, 8.2 μm and 16.5 μm, respectively), while the change from 40 to 80 mmHg constricted vessels by -13 μm (-19 μm, -7.7 μm). Increasing the intraluminal pressure from 80 to 120 mmHg changed the diameter by 1.4 μm (-1.8 μm, 4 μm). Decreasing the intraluminal pressure from 120 to 80 mmHg dilated vessels by 0.6 μm (-2.9 μm, 4.2 μm) (Figure 4).

CM alone did not significantly change the myogenic response (80-40 mmHg: 9.1 μm, 6.2 μm, 10.8 μm, $p = 0.28$; 40-80 mmHg: -9.3 μm, -13.1 μm, -7.1 μm, $p = 0.24$; 80-120 mmHg: -0.1 μm, -4.1 μm, 4 μm, $p = 0.84$; 120-80 mmHg: 1.7 μm, -2.8 μm, 4.3 μm, $p = 0.89$) compared to the control group, respectively (Figure 4).

Vessels treated with blood showed a significant decrease when compared to blood alone.

Some investigators placed blood clots into the brain$^{3,6}$. Other approaches. The most common in vivo techniques are: injection of blood into the brain cisternae and vessel avulsion$^{22,23}$. Some investigators placed blood clots into the brain$^{18}$. In vitro models include application of whole blood$^{89}$ or of vasoactive substances which are hypothetically involved in the development of vasospasm$^{18}$ into the organ bath solution.

In our experience models of SAH using whole blood$^{89}$ were not suitable for the investigation of spontaneous

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**Figure 3. Development of spontaneous myogenic tone during stable intraluminal pressure of 80 mmHg. The data are presented as an average ± SEM. CM: Contrast medium. * indicates a significant difference between curves for groups treated with coagulated blood (volume 2 ml) and without blood, respectively.**
myogenic tone and myogenic response, primarily because whole blood hinders the visualization of pressurized arteries. The use of coagulated blood does not muddle the solution and allow the measurement of vessel diameter. It is possible to use coagulated blood of different ages, over longer periods of time. Importantly, we can control the exact proportion between the volume of the blood clot and PSS surrounding the vessel, and avoid the effects of irregular distribution of blood around the brain cisterns. By careful and controlled isolation of each vessel we can also exclude that vessel dysfunction happens due to mechanical damage by bleeding. Although mechanical irritation of vessels or brain damage are considered as possible contributing factors to clinical vasospasm, in our study we intended to separate the mechanical effects from the effects of blood clot presence and ageing.

We found that exposition to coagulated blood significantly increases the spontaneous myogenic tone of cerebral arteries. This indicates that our \textit{in vitro} model successfully reproduces changes in the vessel tone which correspond to the initial events of vasospasm post SAH \textit{in vivo}. The data support the assumption that the causal agents of vascular spasm originate largely from the clotted blood. We thus believe that the use of coagulated blood immersed in PSS provides a nearer approximation of the environmental conditions in the brain cisterns following SAH in comparison to the models using either whole blood or individual vasoconstrictors.

Cerebral vessels showed an impaired myogenic response in our model of vasospasm. This observation supports clinical evidences that the autoregulation of cerebral blood flow is disturbed after SAH, what may contribute to brain damage. It has been shown that pressurized arteries from rabbits where SAH had been induced \textit{in vivo} have enhanced myogenic response. However, in this study the myogenic response was reversed in both control and spastic vessels when the

\textbf{Figure 4.} Myogenic response quantified as the variation in diameter after controlled changes in intraluminal pressure. CM – contrast medium. (A) Dilation in response to decrease of intraluminal pressure from 80 to 40 mmHg; (B) Constriction in response to increase of intraluminal pressure from 40 to 80 mmHg; (C) Constriction in response to increase of intraluminal pressure from 80 to 120 mmHg; and (D) Dilation in response to decrease of intraluminal pressure from 120 to 80 mmHg. * indicates a significant difference between groups. Non-significant differences between groups are also indicated (n.s.).
intraluminal pressure was increased to what the authors considered as supraphysiological levels (i.e., above 140 mmHg)\textsuperscript{26}. In contrast, in our experiments only the vessels exposed to blood clot showed an impaired myogenic response, and in all levels of pressure. These apparently contradictory findings may result from the use of different models of vasospasm, different time points for investigating the myogenic response during the development of vasospasm, and different protocols for quantifying the myogenic response.

We found out that CM did not significantly influence vessel tone and did not negatively influence the myogenic response, in both healthy as well as in spastic vessels. This is important because patients with SAH have disturbances of cerebral blood regulation\textsuperscript{25}, and many diagnostic and therapeutic procedures needed for their treatment may require the use of CM\textsuperscript{12,13,14}. It has been shown that several types of CM may have potentially deleterious effects on the tone and reactivity of vessels from several vascular beds\textsuperscript{15,16}. Correspondingly, there are evidences that CM affect regional cerebral blood flow in healthy subjects\textsuperscript{17}. Interestingly, Rosengarten et al.\textsuperscript{17} showed that the dynamic cerebral blood flow regulation – the increase of regional cerebral blood flow caused by brain activity – was negatively affected by CM, while stable regional brain blood flow was not affected. We think that these results are compatible with our findings, given that the stable regional blood flow is directly dependent on the myogenic response. Although our results do not show statistical differences for vessels treated with blood clot and CM, we have to consider a small number of samples and multiple comparisons. Moreover, when translating the current findings into \textit{in vivo} condition, we can meet various limitations.

Interestingly, we found that vessels exposed to blood clot and CM showed a lower degree of functional impairment in comparison to vessels exposed to blood clot alone. A possible explanation for this finding could be endothelial damage induced by the CM, with consequent decreased nitric oxide release and increased superoxide production\textsuperscript{15,27}. Although the influence of the endothelium on the myogenic response is usually not marked, some experimental models show that the myogenic response of cerebral arteries might be modulated by the endothelium\textsuperscript{10}.

In summary, we developed a novel, reproducible \textit{in vitro} model of vasospasm post SAH which is adequate to investigate cerebral autoregulation. Our finding that the myogenic response was deranged in our model is compatible with clinical studies showing that the autoregulation of cerebral blood flow is impaired after SAH. There was no negative influence of CM on myogenic tone and myogenic response in cerebral vessels with acute vasospasm. The influence of CM on other mechanisms of regulation of cerebral blood flow in patients with vasospasm and SAH is still unknown, and warrants further investigation using other types of models.

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**References**

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