The effect of propofol on astro- and microglial reactivity in the course of experimental intracerebral haemorrhage in rats

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Abstract
The glial cells play an important role in pathophysiology of the intracerebral haemorrhage (ICH). Thus the attempt at evaluating the possible influence of the propofol on the reactivity of astro- and microglial cells in the course of ICH was performed.

50 rats were divided into two groups depending on the applied anaesthesia. All animals were generally anaesthetized with fentanyl, dehydrobenzperidol and midazolam. No additional agents were given to the animals of the control group (group I). In the experimental group (group II), the animals received additionally intraperitoneally propofol in a dose of 50 mg/kg every thirty minutes. ICH was produced through infusion of the blood into the striatum. The astrocytic and microglial cells population was assessed on the 1, 3, 7, 14 and 21 days after producing a haematoma using antibodies anti-GFAP and OX42. The stereological analysis was applied to estimate the numerical density of immunoreactive cells and the distribution of their types.

On the 14th and 21st days of observation the density of GFAP-immunoreactivity (ir) cells was significantly higher in group II than that in group I. There were no differences in percentage distribution of GFAP-ir astrocytes between group I and group II. On the 3rd, 14th and 21st days of observation the density of OX42-ir cells was higher in group II in comparison with group I. For the 7th, and 21st days of survival the percentage of the ameboid form of OX42-ir cells was significantly lower in group I than that in group II.

The administration of propofol during anaesthesia in the animals with ICH has evoked an increase of the activation of the astro- and microglial cells.

Key words: propofol, astrocyte, microglia, intracerebral haemorrhage, rat.

Introduction
Intracerebral haemorrhage (ICH) is a common and often fatal stroke subtype that leads to a severe neurological damage in survivors with no effective treatment. The current strategy of management of ICH remains controversial. There is no clear indication from the literature of the optimal treatment of ICH surgical or otherwise [5]. Regardless of the applied method, the main goal of treatment of ICH should include control of systemic hypertension and prevention or treatment of the
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Elevated intracranial pressure [23]. Most authors suggest removing of the cerebellar and superficial lobar haematomas [6]. Different methods of surgical treatment of ICH, for instance, open craniotomy [6], the use of endoscopy [3] or a stereotactic injection of thrombolytic agents for liquifying clot and removal [18] have been proposed.

Developing an effective method of treatment means an improved understanding of the pathophysiology of ICH [13]. Recently published investigations direct attention to perihaematomal region as a potential target for therapeutic intervention in the ICH patient [1,7,27,31]. The mechanical injury caused by extravasation of blood leading to reduction of the cerebral blood flow [20,21], inflammatory reaction [8,17] breakdown of hemoglobin [11,37] and complement cascade activation [10,36] have all been implicated as mediators of tissue injury. In these processes the astroglial and microglial cells play an important role. Even minor pathology induces morphological transformation of these cells from resting into activated forms. Activated astrocyte cells synthesize and secrete tumor necrosis factor-alfa, interleukin-1-beta and interleukin-6 [8,20,37]. Activated astrocytes play an important role in formatting the glial scar that is the result of the repair response of the glial [8,20,17]. Ameboid forms of microglial cells are able to migrate [28] and capable of proliferating and phagocytizing [27]. Released form activated microglia tumor necrosis factor-alfa and interleukin-1-beta are responsible for damage of the blood-brain-barrier in the course of ICH [9]. Literature concerning the reaction of astro- and microglial cells in the course of ICH is poorly represented [7,14,15].

Propofol remains a commonly used agent for providing intraoperative anaesthesia as well as sedation in the patient treated in the intensive care unit. Many laboratory and clinical investigations confirm the favorable influence of propofol on intracranial homeostasis [30]. Effects on the cerebral blood flow [16,34], cerebral metabolic rate for oxygen [33] intracranial pressure [22] and neuroprotective property [26,39] give sufficient grounds for recommendation of propofol for neurosurgical procedures.

The proposal of the present study was to investigate whether propofol attenuates reactivity of astroglial and microglial cells in the course of experimental intracerebral haemorrhage in rats.

### Table I. Physiological parameters in groups I and II in the middle stage of production of intracerebral haematoma*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>body weight (g)</td>
<td>300.5±14.3</td>
<td>309.2±13.9</td>
</tr>
<tr>
<td>hemoglobin (g dL⁻¹)</td>
<td>12.73±11.1</td>
<td>11.3±1.7</td>
</tr>
<tr>
<td>glucose (mmol.L⁻¹)</td>
<td>8.11±0.71</td>
<td>7.97±0.97</td>
</tr>
<tr>
<td>temperature (°C)</td>
<td>37.3±0.1</td>
<td>37.2±0.1</td>
</tr>
<tr>
<td>ETCO₂ (kPa)</td>
<td>5.24±0.05</td>
<td>5.26±0.04</td>
</tr>
<tr>
<td>SaO₂ (%)</td>
<td>96.7±0.5</td>
<td>96.5±0.9</td>
</tr>
<tr>
<td>CPP (mmHg)</td>
<td>89.2±4.9</td>
<td>88.9±5.1</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± standard deviation

### Material and methods

50 adult rats (weight 275-350 g; Table I) were used in the study. Animal care and treatment guidelines outlined by the European Community Council Directive of 24 November 1986 (86/609/EEC), as well as the local ethical committee were followed. Animals were divided into two groups depending on the applied anaesthesia. In all the groups, the animals were generally anaesthetized with fentanyl (Fentanyl, Polfa-Warszawa, Poland) with a dose of 0.02 mg.kg⁻¹, dehydrobenzperidol (Droperidol, G .Richter; Hungary) with a dose of 0.75 mg.kg⁻¹ administered intraperitoneally and midazolam maleate (Dormicum, Roche; Switzerland) with a dose of 0.3 mg.kg⁻¹ given intramuscularly every half hour. No additional agents were given to the animals of group I. In group II the animals received additionally intraperitoneally propofol (Diprivan, AstraZeneca; United Kingdom) with a dose of 50 mg/kg every thirty minutes. Each group was divided into five subgroups (five animals in each subgroup) depending on the length of the survival period: 1, 3, 7, 14, 21 days.

After insertion of the intubation tube, the rats were mechanically ventilated with the Small Animal Ventilator SAR 830/p (CWE, Inc. USA), using a mixture of air and oxygen (FiO₂ = 0.5). Parameters of ventilation were adjusted to keep the end-tidal concentration of CO₂ (ETCO₂) constant between 5.19 and 5.45 kPa, and O₂ saturation of the hemoglobin (SaO₂) above 95%. ETCO₂ was monitored with the carbon dioxide analyzer Capstar 100 (CWE, Inc. USA). Continuous monitoring of the SaO₂ was performed with pulse oximeter monitor.
(Novametix; USA) connected with a sensor placed across the animal’s hind foot.

Both the femoral artery and vein were cannulated for continuous mean arterial blood pressure monitoring (MAP), blood sampling for biochemical analysis as well as fluid infusion. MAP was monitored with the direct blood pressure monitor (Stoelting; USA). Maintenance fluids (0.9% NaCl) was administered at the rate of 2.5 ml.kg\(^{-1}\).h\(^{-1}\). Body temperature was maintained at 37.0°C (Table I) using a rectal thermometer and a feedback-controlled heating pad (EST, Stoelting; USA).

The animal was fixed in a stereotaxic frame and than MicroSensor ICP Transducer (Codman Johnson & Johnson Medical Ltd, USA) was inserted epidurally in the parietal region through a separate trepanation hole. For continuous measurement of ICP, the transducer was connected to the Neuro Monitor Interface Control Unit (Codman, Johnson & Johnson Medical Ltd. USA) and a Monitoring System 8000 (Simonsen & Weell; Denmark). A hole was drilled in the frontal bone on the opposite side to the ICP monitoring. After introducing the needle into the striatum (stereotaxic coordinates: B=1.2 mm; L=2.5 mm; H=5.5 mm), 100 µl of autologous blood was injected for 5 minutes and the needle was left in place for another 4 minutes, than it was slowly withdrawn and the skull was sealed.

During the production of haematoma, the plasma levels of glucose and hemoglobin (Table I), were measured using the B-Hemoglobin and B-Glucose Analyzer (HEMOCUE AB, Sweden). Comparability of the physiological condition during the experiments were maintained using continuous monitoring of ETCO\(_2\), SaO\(_2\), and cerebral perfusion pressure (CPP). CPP was calculated from the difference between MAP and ICP. Appropriate results are given in Table I. After the experiment the rats were allowed to awake under control of ETCO\(_2\) and SaO\(_2\). The intubation tube was removed if spontaneous breathing of animals was sufficient (values of ETCO\(_2\) below 5.32 kPa, and SaO\(_2\) above 95%).

**Immunohistochemistry**

The animals were deeply anesthetized with lethal doses of thiopental sodium (Thiopental, Biochemie GmbH, Austria) with the dose of 50 mg.kg\(^{-1}\); then transcardially perfused with 0.9% solution of NaCl, followed by a 4% paraformaldehyde solution in 0.1M phosphate buffer (pH 7.4). The brains were postfixed in 4% paraformaldehyde fixative for 3-4 hours, and then were kept in 0.1 M phosphate buffer containing 15% sucrose (overnight at 4°C) and 30% sucrose (until sunk). Coronal 50-µm-thick serial sections of the brain (five from each animal) were cut with JUNG 1800 cryostat (Leica, Germany). Free floating sections were blocked with 3% NGS containing 0.4% Triton X-100 for 1 hour and then were incubated with the monoclonal antibody (mAb) OX42 recognizing the complement type -3 receptor (CR3/CD11b) (mouse, dilution 1:50, MCA275G, Serotec, Cambrige, UK) and with the primary antibodies anti- GFAP (dilution: 1:250, DAKO, Denmark). Comparability of the immunopositive cells and their morphological type were assessed.

**Qualitative assessment of glial activation**

Structures as well as the localization and morphology of the haematoma were assessed on the sections stained with cresyl violet using Labophot (Nikon, Japan) referring to the stereotactic atlas of the rat brain.

Immunohistochemically labeled sections were analyzed using the fluorescent microscope BX-51 (Olympus, Japan), and confocal laser scanning \(\mu\)-Radiance head (Bio-Rad, United Kingdom) mounted on Eclipse E600 microscope (Nikon, Japan). FITC and Cy3 were excited by a monochromatic beam of 488 nm and 514 nm of length, respectively. 3D stacks of optical confocal scans were obtained by the usage of high quality 20x and 60x oil lenses and optimal iris in LaserSharp 2000 (Bio-Rad, United Kingdom).

The distribution of immunopositive cells and their morphological type were assessed.

**Quantitative assessment of microglial activation**

Intensity of glial reaction was quantified by the number of labeled cells per unit area using C.A.S.T. Grid stereological system (Olympus; Denmark). The
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A reference area bordered by the ventricle, corpus callosum, external capsule and the level of rhinal sulcus with the exclusion of the haematoma region were marked and measured under the small magnification. Cells were counted in test frames (of area equal to 0.2765 mm²) chosen in a systematic random fashion. As a minimum one third of the reference area was assessed. On the basis of their morphology astroglial and microglial cells were classified into resting (Fig. 1A, C) and activated (Fig. 1B) or ameboid (Fig. 1D) types. The data were then transferred to the spreadsheet; the number of cells per unit area was calculated. Finally, the mean value of the numerical density of glial cells as well as the percentage distribution of their types in each case were estimated.

Fig. 1. Types of astroglial and microglial cells: resting form of astrocyte (A), activated astrocyte (B), resting form of microglia (C), ameboid form of activated microglia (D). Staining A-B-GFAP, C-D-OX42. Magnification bar=50 µm
The statistical analysis was done by means of Statistica v. 6.0 (Statsoft; USA) and GraphPad Instat (Graph Pad Inc.; USA). The significance of differences of values of body weight, glucose, hemoglobin, temperature, ETCO₂, SaO₂, as well as CPP between groups I and II were assessed by the Mann-Whitney test. The influence of the anaesthetic agent and period of survival on the numerical density and percentage distribution of glial cells were tested using the analysis of variance followed by the LSD test for the planned study of differences between groups and subgroups. 95% confidence intervals for the differences between means were calculated.

Results

In group II significant differences in the density of GFAP-ir astrocytes were observed between the 7th and 14th and 14th and 21st days of survival (Fig. 2, Tab. II). On the fourteenth and twenty first days after production of haematoma statistically significant differences in the numerical density of GFAP-ir astrocytic cells between groups I and II were noted (Fig. 2, Tab. II). In group I there was an increase in per cent distribution of activated GFAP-ir cells that remained up till the end of the observation (Fig. 3, Tab. II). In group II there was an increase in per cent distribution of activated GFAP-ir cells between the 1st and 3rd that decreased between the 3rd and 7th and than again increased between the 7th and 14th days after production of haematoma (Fig. 3, Tab. II).

In group I there was an increase of density of OX42-ir microglia only between the 3rd and 7th observation days whereas in group II an increase of the number of OX42-ir per mm sq. was found between the 7th and 14th observation days, and then a decrease between the 14th and 21st days after production of haematoma (Fig. 4, Tab. III). On the third, fourteen and twenty first observation days differences in the numerical density of OX42-ir microglia between groups I and II was observed (Fig. 4, Tab. III).

Table II. Significant differences between the means along with 95% confidence intervals (CI) and p-value of the LSD test (p) for analyzed parameters of GFAP-ir astrocyte

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Groups</th>
<th>Survival day</th>
<th>Difference</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>numerical density of astroglia</td>
<td>group II</td>
<td>7 vs 14</td>
<td>-113.132</td>
<td>-168.392</td>
<td>-57.872</td>
<td>0.000184</td>
</tr>
<tr>
<td>numerical density of astroglia</td>
<td>group II</td>
<td>14 vs 21</td>
<td>111.449</td>
<td>52.09</td>
<td>170.808</td>
<td>0.000507</td>
</tr>
<tr>
<td>numerical density of astroglia</td>
<td>group II vs group I</td>
<td>14</td>
<td>158.31</td>
<td>103.05</td>
<td>213.57</td>
<td>0.000001</td>
</tr>
<tr>
<td>numerical density of astroglia</td>
<td>group II vs group I</td>
<td>21</td>
<td>91.211</td>
<td>22.669</td>
<td>159.753</td>
<td>0.010453</td>
</tr>
<tr>
<td>per cent of activated form</td>
<td>group II</td>
<td>1 vs 3</td>
<td>-88.8313</td>
<td>-95.0398</td>
<td>-82.6229</td>
<td>0.000012</td>
</tr>
<tr>
<td>per cent of activated form</td>
<td>group II</td>
<td>3 vs 7</td>
<td>10.4696</td>
<td>3.6686</td>
<td>17.2706</td>
<td>0.000479</td>
</tr>
<tr>
<td>per cent of activated form</td>
<td>group II</td>
<td>7 vs 14</td>
<td>-7.461</td>
<td>-13.2408</td>
<td>-1.6813</td>
<td>0.012779</td>
</tr>
<tr>
<td>per cent of activated form</td>
<td>group I</td>
<td>1 vs 3</td>
<td>-85.575</td>
<td>-94.3551</td>
<td>-76.7949</td>
<td>0.00031</td>
</tr>
</tbody>
</table>
In both groups there were increases of per cent of ameboid form of OX42-ir microglia between the 3rd and 7th and between 7th and 14th and then decreases between the 14th and 21st days of observation (Fig. 5, Tab. III). There were also found the differences of per cent of ameboid form of OX42-ir microglia between groups I and II on the 7th and 21st survival days (Fig. 5, Tab. III).
Discussion

In the pathology developing in cases of ICH, a prominent role is played by astroglial and microglial cells. We have studied the effect of propofol on the reactivity of astrocytes and microglia in the course of experimental ICH. Applying an experimental model is similar to the real condition of the intracerebral haematoma formation. In animals anaesthetized with the use of propofol, an increased and extended activation of both GFAP-IR astrocytes and OX42-IR microglia was found.

The clinical study suggests that delayed brain edema (in the 2nd and 3rd weeks after the onset of ICH) is due to a breakdown of hemoglobin induced impairment of the blood-brain barrier [11]. However, Zazulia et al. [40] have proposed other, more speculatively pathophysiological clarification for this late edema formation. From 1 to 14 weeks after ICH, new vessels around haematoma were created. These vessels are devoid of a fully developed blood-brain barrier. Moreover, animal models demonstrated that propofol decreases both cerebral blood flow and cerebral metabolic rate for oxygen [2,34]. Some of the animal investigations have suggested that the decrease in cerebral blood flow is greater than the decrease in cerebral metabolic rate for oxygen, that may lead to a critical drop of cerebral perfusion pressure [2,34]. Several clinical studies found a frequent incidence of jugular bulb oxygen saturation values compatible with global cerebral hypoperfusion in patients operated on brain tumors in anaesthesia with the use of propofol [12,19]. Especially, the defective blood-brain barrier may be particularly sensitive to ischemic injury evoked by propofol. This may in part explain our findings.

It may be presumed that astroglial and microglial activations have no direct connection with the injury evoked by intracerebral haemorrhage. Lipid emulsion used as a carrier for propofol may be responsible for glial activation in our investigation. Weight et al. [32] demonstrated that lipid emulsions activated N-methyl-D-aspartate (NMDA) receptors channel. Lipid mediators potentiate NMDA receptor activity [29]. Up-regulation of microglial cell throughout the brain after an intravenous injection of lipopolysaccharide (LPS) was observed by Buttini et al. [4] and Xu et al. [38]. Moreover, LPS released from the outer membrane of Gram-negative bacteria during sepsis may also lead to activation of microglial cells [25].

Propofol evokes a neuroprotective effect, which is apparently mediated by restoration of the glutamate uptake by astrocytes impaired during injury [35]. On the other hand, lipid emulsion stimulates NMDA receptors, that leads to injury. This speculative theory may in part explain our findings especially when we take into account the fact that in some experiments pure substance of propofol (without lipid carrier) was used [35].

In sum, these findings provide evidence for activation of astroglial and microglial cells by propofol. The ability of propofol for modulation of the inflammatory reaction may have a clinical implication. Activation of glial cells in the central nervous system plays a key role during injury and neurodegeneration. Therefore elucidation of the molecular mechanism of our outcome may have a crucial meaning for basic and clinical neurological research.

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References