Recent experimental studies in rodents suggest that treatment with inhibitors of phosphodiesterase type 5 (PDE5) (tadalafil, sildenafil, zaprinast) not only increases cerebral blood flow but also improves functional recovery after stroke. Here, we investigated in a mouse model of stroke the effects of vardenafil on survival, functional outcome and lesion size after experimental stroke. Mice were subjected to experimental stroke by occlusion of the middle cerebral artery (MCAO) for 45 min. A group of mice received vardenafil (twice 10 mg/kg body weight per day orally over 14 days) starting 3 h after MCAO. Control animals received the vehicle only. Survival, body weight, and behavior were monitored over 4 weeks and brain lesions were measured by T2-weighted MRI, hematoxylin/eosin — as well as GFAP-staining of cryostat sections, subsequently. The mortality in MCAO-operated animals amounted to 45% until day 10 after stroke and no significant difference in survival between the vardenafil- and vehicle-treatment groups was observed. Compared to sham-operated animals, MCAO-operated mice from both treatment groups demonstrated a significant weight loss until day 5 and regained their body weight by day 14 after ischemia. There was no significant difference in survival between the vardenafil- and vehicle-treated MCAO groups. In behavioral studies (sucrose consumption and pole test), analyzing sensorimotor functions as well as a parameter of depression-like symptoms, we observed no significant effect of vardenafil treatment on functional recovery in our model of stroke. Although we observed a trend towards less hemispherical atrophy in the vardenafil compared to the vehicle-treated group four weeks after MCAO our data do not suggest a functionally relevant CNS-tissue protective or regenerative effect in murine stroke.

© 2009 Elsevier B.V. All rights reserved.
1. Introduction

Recent experimental studies in rodents suggest that treatment with inhibitors (tadalafil, sildenafil, zaprinast) of phosphodiesterase type 5 (PDE5) not only increases cerebral blood flow and angiogenesis in the ischemic penumbra but also promotes functional recovery and neurogenesis after stroke (Gao et al., 2005; Zhang et al., 2002, 2003, 2005). Sildenafil at doses of 2 or 5 mg/kg per day increased brain levels of cGMP, augmented neurogenesis, and reduced neurological deficits when given to rats orally for 7 consecutive days starting 2 or even 24 h after stroke (male Wistar rats, embolic middle cerebral artery occlusion). However, there was no significant difference in infarct volume among the experimental groups. Concluding from this data, PDE5 inhibition may have a role in promoting recovery from stroke (Zhang et al., 2003). Functional recovery due to sildenafil treatment was observed in young as well as in aged rats following embolic stroke. However, effective doses reported in Zhang et al. (2005) were higher in aged (10 mg/kg daily) than in young animals (2 mg/kg).

It was our aim to test whether the PDE5 inhibitor vardenafil improves outcome after experimental stroke. As primary endpoint we inquired whether inhibition of PDE5 by vardenafil within a therapeutically relevant time window improves functional recovery after transient focal cerebral ischemia. As secondary endpoint, we tested whether inhibition of PDE5 by vardenafil within a therapeutically relevant time window affects brain lesions four weeks after transient focal cerebral ischemia. We used our well accepted murine middle cerebral artery occlusion (MCAO) model (Prass et al., 2003a). This model mimics the most common and most relevant subtype of ischemic stroke in humans — territorial infarction in the middle cerebral artery (Mergenthaler et al., 2004). As outcome measure, we analyzed neurological deficits and brain lesion size measured by volumetry based on MRI, histology and immunohistochemistry.

2. Results

2.1. Functional recovery

Experimental stroke was induced by 45 min occlusion of the middle cerebral artery (MCAO). Starting 3 h after surgery, animals were treated twice daily with vardenafil (10 mg/kg orally) for 14 days (Fig. 1). With respect to the initial functional deficit, there was no significant difference between vardenafil- and vehicle-treated animals until day 1 after stroke (Fig. 2). All sham-operated controls exhibited no functional deficit (data not shown).

As outcome measure we first studied mortality and body weight. It is well known that mortality in this mouse model is up to 56% within the first 7 days (Meisel et al., 2004). In this study, the mortality in MCAO-operated animals amounted to 45% until day 10 after stroke and no significant difference in survival between the vardenafil- and vehicle-treatment groups was observed (Fig. 3a). Since mortality affected both groups to the same degree and thus is not expected to introduce a relevant bias, we excluded the animals that have died from further data analysis. Compared to sham-operated animals, MCAO-operated mice from both treatment groups demonstrated a significant weight loss of (mean±95% confidence interval) 22±10% (vardenafil group) and 17±5% (vehicle group) until day 5, respectively. In both groups, surviving animals had almost regained their body weight by day 14 after ischemia (Fig. 3b). However, there was no significant difference between vardenafil and vehicle-treated MCAO groups.

The primary endpoint of our study was the effect of vardenafil on functional recovery in our murine stroke

![Fig. 1 – Experimental paradigm.](image)

![Fig. 2 – Neurological deficit 1 day after MCAO in vardenafil- and vehicle-treated animals. Mice (n=20 in each group) were MCAO operated and received either vardenafil or vehicle as described in Experimental procedures. Neurological deficits were assessed as described in Experimental procedures. Data are given as cumulative percentage of mice in each group with the respective neurological deficit score (see legend and Experimental procedures).](image)
model. Functional outcome was measured by using behavioral tests (pole test, wire hanging test) starting at 14 days after experimental stroke. Vehicle-treated animals and sham-operated animals served as controls (Fig. 1). Pole test was performed on day 19 post MCAO (Figs. 4a and b). Animals of both MCAO groups needed more time to turn head down (t turn) and to reach the floor (t floor) compared to mice from sham operated groups ($p=0.006$, $p<0.001$ respectively). However, vardenafil treatment did not cause any significant effect on both parameters, neither in the MCAO nor in the sham group, although the MCAO group treated with vardenafil shows a small non-significant trend towards better performance. The wire hanging test (Fig. 4c) did not reveal a difference between MCAO and sham operated animals, and thus could not be used to determine functional outcome after ischemia. In conclusion, though relevantly impaired by MCAO, results from pole test did not reveal a long-time effect of vardenafil in functional recovery.

2.2. Brain lesion sizes

To determine whether vardenafil affects brain tissue damage after experimental stroke (45 min MCAO), the long term lesion size was measured four weeks after cerebral ischemia based on T2-weighted MRI as well as HE staining and GFAP staining of coronal brain cryostat sections. Additionally, a computer-assisted hemisphere volumetry was performed, based on T2-weighted MRI and HE-stained coronal brain cryostat sections. Sham-operated animals served as controls and did not show any lesions (data not shown).

![Graph](image)

**Fig. 3** – MCAO induced mortality and weight loss is not reduced by vardenafil treatment. (a) Both vehicle and vardenafil treated animals showed a 45% mortality within 10 days after MCAO surgery ($n=10$ for sham treated groups, $n=20$ for MCAO treated groups). (b) Compared to sham operated animals surviving animals of both MCAO groups showed a pronounced weight loss with a body weight minimum at days 4–6 post MCAO. There was no significant difference between the vardenafil treated and the vehicle group (error bars=95% confidence interval).

![Graph](image)

**Fig. 4** – Functional recovery after cerebral ischemia. During pole test (performed on day 22 post MCAO), both the time required to turn completely head down (a) and the time needed to reach the floor (b) were significantly increased in the MCAO groups compared to sham groups ($p<0.001$, $p<0.0001$, respectively, Wilcoxon rank sum test). However, treatment with vardenafil had no significant effect on both parameters. Wire-hanging test (c, day 19 post MCAO) revealed no difference between groups regardless of drug treatment or surgical intervention.
T2-weighted MRI, HE-, and GFAP-stained cryostat sections showed lesions and signs of atrophy in the hemisphere ipsilateral to MCAO surgery for both vehicle (Fig. 5) and vardenafil treated animals (data not shown). The lesions measured by T2-weighted MRI include areas filled with cerebrospinal fluid and therefore appear larger compared to HE-histology based volumetry. The lesion volumes in T2-weighted MRI are significantly smaller in the vardenafil-treated group compared to the vehicle group (Fig. 6a, \( p<0.05 \)). This difference was not apparent in histological measurements: brain lesion volumetry based on HE-staining neither demonstrates a significant difference between treatment groups (Fig. 6b) nor correlates well with lesions in T2-weighted MRI (Fig. 6c). When performing an atrophy measurement of the affected hemisphere (see Experimental procedures section) the relative atrophy of the affected hemisphere (ipsilateral to MCAO surgery) shows a good correlation between MRI and histological measurement (Figs. 6d-f). Both methods demonstrate a trend toward less hemispherical atrophy in the vardenafil group compared to vehicle-treated mice. Although non-significant, these data suggest that vardenafil exhibits either CNS-tissue protective or regenerative properties. However, glial scar volume based on GFAP-staining demonstrated no significant difference between both treatment groups and T2-weighted MRI might appear normal despite pronounced gliosis (Figs. 7, 8).

Fig. 5 – Lesion four weeks after MCAO (vehicle treatment). (a) T2 weighted MRI of an animal from the vehicle group. Left sided MCAO for 45 min caused a cortical-subcortical lesion extending over the whole MCAO territory. (b) Histological hematoxylin and eosin-stained cryostat sections of the same animal. (c) Immunohistological section after staining for GFAP. The left hemisphere shows signs of glial scar and atrophy caused by MCAO.
2.3. Effect of vardenafil on cerebral blood flow after experimental stroke in mice

Reperfusion CBF was measured in a separate group of animals using FAIR MRI (Leithner et al., 2008; Prass et al., 2007; Prüss et al., 2008). As expected, we demonstrated a reduction of ipsilateral hemispheric CBF in the MCAO-treated animals. However, vardenafil treated animals did not show a significantly better CBF in the reperfusion phase (Fig. 9). During FAIR MRI measurements mean arterial blood pressure and body temperature were kept within physiological values (Table 1).

3. Discussion

Failure to reject the Null hypothesis, e.g. vardenafil treatment is not superior compared to vehicle (“placebo”) treatment, does not prove that the Null is correct. The probability that the Null was not rejected, although it was actually false, is measured by \( \beta \) (Power = 1 - \( \beta \)), and the associated error is termed type II error. In the independent tests performed (infarct volume, weight change, pole test, T2 weighted MRI, FAIR MRI), standard deviations ranged from 20 to 50% of the mean. Such SDs reflect the high physiologic variability, as well as the variance within the individual assay, and are well within published standards. Power analysis was the basis for the current study protocol, and is only valid a priori (Aberson, 2002; Dirnagl, 2006). If used for a priori analysis, the current data would result in effect size thresholds at error levels of \( \alpha = 0.05 \) and \( \beta = 0.8 \) of around 20–40% of the mean. However, one must consider that five different, and independent (not with regard to the animal, but with regard to the assay) measures were taken. The fact that all functional and structural parameters were not significantly different in vardenafil treated vs. control animals, (except for T2 weighted MR at 4 weeks after MCAO), point to a robust finding, with also formally (but hard to quantify) smaller detectable effect sizes than the ones given above for univariate analysis.

This study’s primary aim was to demonstrate that the PDE5 inhibitor vardenafil promotes functional recovery after experimental stroke. The applied behavioral tests do not lend support to this hypothesis. These tests monitor different aspects of functional impairment in the applied mouse model. The wire hanging test did not show significant changes between MCAO and sham operated animals and thus could not evaluate differences in functional recovery from MCAO in this study. It relies on the animal’s ability to suspend its body on a steel wire elevated 60 cm above the floor. The performance of a mouse in this test is considered as a correlative of the animal’s grasping ability, requiring grip strength as well as endurance. In previous experiments using Sv129 mice we demonstrated a robust effect of experimental stroke on the animal’s performance in the wire-hanging test compared to sham controls as late as five weeks post MCAO.
establishing wire-hanging as a reliable test in behavioral studies of murine stroke. More importantly, using the wire-hanging test we were able to observe the beneficial effects of training on outcome four weeks after cerebral ischemia (Gertz et al., 2006). In this study however, the wire-hanging test failed to reveal any difference between sham controls and MCAO animals 19 days after surgery. In addition, we observed no effect of treatment. This might be explained by a superior sensory–motor recovery potential of the C57 mouse strain compared to the Sv129 strain.

The original pole test (Ogawa et al., 1985) was employed to quantify bradykinesia in models of Parkinson’s disease (Matsuura et al., 1997). Experimental MCAO-induced stroke causes up to 50% depletion of striatal dopamine (Winter et al., 2005). In our study, animals of both MCAO groups needed more time to turn head down (t turn) and to reach the floor (t floor) compared to mice from sham operated groups. Though therefore suitable to assess long term functional deficits from MCAO, the pole test did not reveal any significant effect of vardenafil treatment. In addition, the sucrose consumption test was used to determine the hedonic state of the mouse, which is known to be reduced by MCAO induced stroke (Craft and DeVries, 2006). We observed a significant reduction in sucrose consumption at days 16 and 17 after surgery in MCAO-treated animals compared to the sham operated control group. However, no significant effect of vardenafil treatment was observed (data not shown).

Taken together, our data did not reveal a significant effect of long term treatment with vardenafil on functional recovery after MCAO. In addition, vardenafil did not reduce post-stroke mortality and loss of body weight. From the vehicle-treated sham animals two died in the course of the experiment. Since these animals died 5 days after sham-operation we speculate that this is due to infections caused by gavage using a tube. Though the mortality in MCAO-group was as expected from previous studies (Prass et al. 2003b; Meisel et al. 2004), we cannot exclude that gavage for drug administration also influenced mortality in the MCAO-groups.

These findings are in contrast to studies demonstrating that treatment with PDE5 inhibitors (tadalafil, sildenafil) improves functional recovery after rat cerebral ischemia (Zhang et al., 2002, 2006). These authors observed a reduced body weight loss and improved performance in foot-fault test and adhesive removal test in PDE5 inhibitor treated stroke animals. Tadalafil (2 or 10 mg/kg bw per day) and sildenafil (2 or 5 mg/kg bw per day) were administered orally for 6 and 7 days starting 24 h after stroke onset.

In principle, our negative data do not necessarily exclude a positive effect of vardenafil on functional outcome after stroke. A false negative result might have several reasons. (1) The vardenafil administration (dose or treatment regimen) might have been inappropriate to reach sufficient drug concentrations in serum and brain. Although we have not measured vardenafil tissue concentrations in our study, this seems unlikely, since we followed an established and proven protocol which was effective in other disease models. It has been shown to be effective in many animal models for glomerulonephritis (Hohenstein et al., 2008), bladder function (Filippi et al., 2007) and benign prostatic hyperplasia (Tinel et al., 2006). In mice, vardenafil has been shown to be effective when given at 1/5 of the dosage of sildenafil in a study on cystic fibrosis (Lubamba et al., 2008). Considering effective dosages in sildenafil stroke studies (2 to 10 mg/kg) this would correspond to 0.4 to 2 mg/kg. With the chosen dosage of 10 mg/kg we are confident to compensate for possible effects of a faster metabolism in mice. In addition, we found significant differences between vardenafil and vehicle treatment groups in T2 weighted MRI lesion size and a trend towards less brain atrophy four weeks after stroke (see below), demonstrating a biological effect of treatment. (2) One might speculate that differences in species or stroke model could explain the result discrepancy between our and other groups. To our knowledge, an improved functional post-stroke recovery induced by PDE5 inhibitor treatment was published so far only by one group studying a rat model of embolic stroke (Zhang et al., 2002, 2006). Despite the well known fact that behavioral testing in mice is rather difficult compared to rats, our behavioral data demonstrate a significant functional impact of stroke even four weeks after stroke (Figs. 4a–c). Therefore, it appears unlikely that these tests failed to reveal a relevant difference in functional recovery in the MCAO mouse model.
The PDE5 inhibitors tadalafil and sildenafil were demonstrated to enhance neurogenesis, increasing the numbers of proliferating neural progenitor cells in the ischemic penumbra (Zhang et al., 2002, 2006). In addition, using the same rat model of stroke, sildenafil was demonstrated to enhance angiogenesis in the ischemic brain (Li et al., 2007). However, neither sildenafil nor tadalafil reduced the infarct volume significantly, measured by MRI or HE staining 1 to 6 weeks and 4 weeks, respectively (Li et al., 2007; Zhang et al., 2002, 2006). We here demonstrated a significantly reduced lesion size measured by T2-weighted MRI in vardenafil-treated compared to vehicle-treated mice four weeks after MCAO. In addition, we observed a trend towards smaller lesion sizes in vardenafil-treated animals measured by H&E or GFAP-staining as markers of overall tissue demise and reorganization, or glial scarring respectively. Furthermore, our data suggests that there is more intact brain parenchyma ipsilateral to MCAO in the vardenafil-treated group compared to the control group: hemispheric atrophy four weeks after cerebral ischemia tended to be smaller, for histological as well as MRI based measurements. Thus, we speculate that vardenafil exhibits tissue regenerative effects.

Guanosine 3′,5′-cyclic monophosphate (cGMP) is an important regulator mediating a vasodilatory effect on cerebral

Fig. 8 – Normal T2-weighted brain MRI with a pronounced glial scar 4 weeks after MCAO. (a) T2 weighted MRI of an animal from the vardenafil group. Cortical and subcortical tissue has recovered after 45 min of MCAO with no apparent atrophy. (b) Histological HE-stained cryostat sections of the same animal showing no apparent brain lesion. (c) GFAP-staining reveals a pronounced subcortical glial scar in the hemisphere ipsilateral to the MCAO surgery.
vessels. PDE5 is highly specific for the hydrolysis of cGMP and involved in the regulation of cGMP signaling. Therefore, inhibitors of PDE5 cause intracellular accumulation of cGMP. Since enhanced cGMP levels are known to cause dilation of cerebral vessels, PDE5 inhibitors are thought to improve cerebral blood flow in the penumbra of the ischemic brain tissue, thereby saving tissue at risk. In fact, using a rat model and laser Doppler flowmetry, one group demonstrated that the 5PDE inhibitor zaprinast (10 mg/kg bw iv) increased relative cerebral blood flow in the ischemic brain by ≈20% when administered 10 min after initiation of a permanent MCAO (Gao et al., 2005). In addition, 4 h after MCAO lesion volume was significantly decreased by 21% compared to the control group. To test whether vardenafil augments reperfusion after MCAO, we measured CBF with FAIR MRI. In previous studies we have shown that this method can detect significant differences in hemispheric reperfusion (Leithner et al., 2008; Prass et al., 2007). At 330 min post MCAO, we demonstrated a significant CBF reduction in the hemisphere ipsilateral to the MCAO. However, there was no effect of vardenafil on CBF in both the ischemic as well as in the non-ischemic hemisphere. Thus, our data are in concordance with clinical data demonstrating that the 5PDE inhibitor sildenafil has no effect on CBF in humans, as determined by SPECT and ultrasound (Kruuse et al., 2002, 2003). In contrast, laser Doppler derived relative CBF in rats was reported to increase by ≈50% after administration of sildenafil (Zhang et al., 2002). However, in this study, the authors point out that this effect was too short termed to be responsible for the improved functional recovery in their rat stroke model. In a further study, this group looked at significant differences of regional CBF (based on continuous arterial spin labeling MRI measurements) between boundary and core regions of focal ischemia, and were able to identify two time points (3 weeks and 6 weeks post MCAO) in which sildenafil treated animals exhibited a better relative perfusion (boundary region vs. core region) than control animals. However, these long-term results occurring weeks after the initial stroke were not ascribed to vasodilatory actions of PDE5 inhibitors. Rather, an increased angiogenesis was postulated (Li et al., 2007). Taken together, the effect of PDE5 inhibitors on CBF might be species dependent: whereas data from the aforementioned rat model seems to point into the direction of augmented perfusion and/or angiogenesis, the data from our study and studies on human subjects do not support a relevant CBF enhancement by PDE5 inhibition.

In conclusion, we found no evidence that long-term treatment with the PDE5 inhibitor vardenafil improves functional outcome after experimental stroke. Since hemispheric atrophy four weeks after cerebral ischemia tends to be smaller in vardenafil- compared to vehicle-treated animals, one might speculate that vardenafil exhibits tissue regenerative or protective functions despite the negative data in behavioral testing. In order to proof the later finding further preclinical studies are indispensable.

Table 1 – Physiological variables during CBF measurement

<table>
<thead>
<tr>
<th>Group</th>
<th>MABP [mm Hg]</th>
<th>Body temperature [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>102±13</td>
<td>37.1±0.3</td>
</tr>
<tr>
<td>Vardenafil</td>
<td>100±12</td>
<td>37.3±1.4</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD (n=5/group). MABP, mean arterial blood pressure, calculated from MABP=DBP+1/3*(SBP−DBP) where DBP and SBP are diastolic and systolic blood pressure, respectively.

4. **Experimental procedures**

4.1. **Animal model of stroke**

For all experiments, we used 7-week-old male C57BL/6 mice weighing 18 to 22 g. All experiments were performed and quantified in a randomized fashion by investigators blinded to treatment groups. Treatment of animals was done by a separate technician after randomization, and animals were reassigned to groups by number codes after completion of analysis. All surgical procedures were approved by the local authorities. The surgical procedure of MCAO did not exceed 10 min and was performed as described previously (Prass et
Briefly, a monofilament was introduced into the common carotid artery under halothane narcosis, advanced to the origin of the MCA, and left there for 45 min until reperfusion. Occlusion and reperfusion were verified by laser Doppler flowmetry (Peri Flux 4001 Master, Perimed). During surgery and MCAO, rectal temperature was maintained between 37.0 °C and 37.5 °C with a heating pad. Mice were kept in heated cages for the next 2 h, and rectal temperature was frequently measured. Animals were then returned to their home cages and allowed free access to food and water.

4.2. Drug administration

Treatment started at 3 h post MCAO and was maintained for a period of 14 days. Vardenafil (10 mg/kg every 12 h), dissolved in vehicle (500 μl 96% ethanol, 2500 μl Solutol HS15, 2500 μl H2O) was administered orally by gavage. Animals were randomized and assigned to one of four treatment groups: 1. sham operated control group treated with a vehicle, 2. sham-operated control group treated with vardenafil, 3. MCAO control group treated with vehicle, 4. MCAO group treated with vardenafil.

4.3. Monitoring and behavioral assessment

Over 14 days after MCAO or sham surgery mice were monitored for survival and body weight. The course of body weight was determined as a measure of general stress induced by cerebral ischemia and recovery from it. On day 1, neurological deficit was assessed by a modified Bederson score as described previously (7,8): 0=no deficit, 1=decreased extension of forepaw, 2=circling, 3=loss of postural reflexes, 4=death). Three weeks after MCAO or sham surgery the animals were subjected to two different behavioral tests. The wire hanging test (day 22) was performed according to Matsuura et al. (1997) and Korpi et al. (1999), with minor modifications. The test apparatus consists of a vertical steel pole covered with a tape (Durapore) to create a rough surface. Each mouse was trained on the day prior to testing. The mouse was placed head upward on the top of the pole and then allowed to descend down. The time until the mouse turned completely head downwards (t turn) and the total time to descend down and reach the floor with its four paws (t floor) were recorded. If the animal was unable to turn completely, the time to reach the floor was also attributed to t turn. Each animal was tested on 5 trials, the average score was taken as the final pole test score.

4.4. Measurement of brain lesions with MRI

Four weeks after MCAO or sham surgery MRI was performed on a Bruker 7 T PharmaScan® 70/16 with a Bruker 98/38 mm RF Coil, operating on Paravision software platform (Bruker, Karlsruhe, Germany). Mice were anesthetized with 1.5% isoflurane in an oxygen/air mixture. They were then fixed using a stereotactic frame and positioned in the magnet bore. T2 weighted MRI was achieved with a TurboRARE sequence (imaging parameters: 256 x 256 in plane resolution, 20 slices with a thickness of 500 μm, FOV 28.5 mm, TR 3500 ms, TE 56 ms, acquisition time 6 min). The axial slices were chosen to cover the region between the olfactory bulb and the cerebellum. In post hoc analysis the size of the lesion apparent in the T2 weighted imaging was determined using custom written imaging processing tools programmed with MATLAB (The Mathworks, Inv, Natick, MA).

4.5. Measurement of brain lesions based on histological and immunohistological assessment

Brains were removed after MRI measurement (28 days after MCAO/sham surgery), snap-frozen, and stored at −70 °C. Lesion areas were quantified in 20 μm hematoxylin and eosin–stained (HE) cryostat sections by numeric integration of areas of marked pallor times section distance (Meisel et al., 2004; Prass et al., 2003b). Since lesions in T2-weighted MRI and lesions in histological measurement differed (see Results section), an additional post-hoc analysis of relative hemisphere atrophy was performed. The area of the delineated lesion and the ventricle areas were subtracted from the brain slice images. The remaining volume of the left (MCAO treated) hemisphere was divided by the volume of the right (intact) hemisphere to obtain the relative hemisphere atrophy. In order to further assess brain lesion based on the glial scar we performed GFAP staining. Cryostat sections of 12 μm were fixed in 4% paraformaldehyde for 10 min, and immunohistochemical analysis was performed as described (Lehnardt et al., 2007) by using antibodies against GFAP (obtained from Dako, Carpinteria, CA). Brain lesions were quantified by numeric integration of areas of marked GFAP staining times section distance.

4.6. Measurement of cerebral blood flow with FAIR MRI

In a separate group of animals, the effect of vardenafil on the acute reperfusion after MCAO was studied by measuring hemispheric cerebral blood flow (CBF) with FAIR MRI. In this group, MCAO was performed as described above. At 180 min after MCAO the animals were treated with a single dose of either Vardenafil (10 mg/kg) or vehicle, administered by gavage. 330 min after MCAO, CBF measurement with FAIR-MRI (same 7 T MRI scanner as above) was performed as described (Leithner et al., 2008; Prass et al., 2007). Mice were anesthetized with etomidate (bolus of 25 mg/kg body weight). They were then fixed using a stereotactic frame and positioned in the magnet bore. Body temperature was monitored with an MR-compatible physiology monitoring unit and maintained within physiological limits using a heated water jacket. Arterial blood pressure was measured periodically (every 10 min) with a tail cuff pneumatic device (XP10000, Kent Scientific Corporation, Torrington, USA). For FAIR MRI, five neighboring slices at 2 mm distance between olfactory bulb and cerebellum were selected. For quantitative analysis, each slice was divided into two horizontal symmetrical halves, rendering the mean hemispheric CBF for each animal.
4.7. Statistical analysis

Differences in lesion size, Bederson score, hemispheric CBF and behavioral assessment (pole test, sucrose consumption) were tested for significance by applying Wilcoxon rank sum test (p < 0.05 was considered significant).

Acknowledgments

This work was supported by the Helmholtz Gemeinschaft für Forschungseinrichtungen, the Deutsche Forschungsgemeinschaft, the Hermann und Lilly Schilling Stiftung, the VolkswagenStiftung, the Bundesministerium für Bildung und Forschung (Center for Stroke Research Berlin), and the Bayer Vital GmbH.

REFERENCES