Improvement in Microregional Oxygen Supply/Consumption Balance and Infarct Size After Cerebral Ischemia-Reperfusion With Inhibition of p70 Ribosomal S6 Kinase (S6K1)

Harvey R. Weiss, PhD, Scott J. Mellender, MD, Geza K. Kiss, MD, Xia Liu, MD, and Oak Z. Chi, MD

Background: We tested the hypothesis that inhibition of p70 ribosomal S6 kinase (S6K1) would decrease infarct size and improve microregional O_2 supply/consumption balance after cerebral ischemia-reperfusion. *Methods:* This was tested in isoflurane-anesthetized rats with middle cerebral artery blockade for 1 hour and reperfusion for 2 hours with or without PF-4708671 (S6K1 inhibitor, 75 mg/kg, 15 minutes after blockade). Regional cerebral blood flow was determined using a C^{14}-iodoantipyrine autoradiographic technique. Regional small vessel (20-60 μm diameter) arterial and venous oxygen saturations were determined microspectrophotometrically. *Results:* There were no significant hemodynamic or arterial blood gas differences between groups. The control ischemic-reperfused cortex had a similar O_2 consumption to the contralateral cortex. However, microregional O_2 supply/consumption balance was significantly reduced in the ischemic-reperfused cortex with many areas of low O_2 saturation (23 of 80 veins with O_2 saturation below 45%). PF-4708671 did not significantly alter cerebral blood flow or O_2 consumption. However, it significantly reduced the number of small veins with low O_2 saturations in the reperfused region (6 of 80 veins with O_2 saturation below 45%). This was associated with a significantly reduced cortical infarct size after S6K1 inhibition (12.9 ± .8% control versus 6.6 ± .3% PF-4708671). *Conclusion:* This suggests that S6K1 inhibition is important for cell survival and that it reduces the number of small microregions with reduced local oxygen balance after cerebral ischemia-reperfusion.

Key Words: Cerebral ischemia-reperfusion—cerebral oxygen supply/consumption—S6K1—brain protection

Introduction

Early restoration of regional cerebral blood flow is the primary means of treatment for cerebral ischemia. It is usually beneficial and reduces ischemic brain injury.[1,2] However, restoration of blood flow may not always improve neurologic outcome because restoration of blood flow to an ischemic area may lead to some pathophysiologic changes in that region.[3] In addition, cerebral blood flow, glucose utilization, and O_2 consumption may or may not be fully restored after reperfusion.[4-6] Microregional O_2 supply/consumption balance is reduced and heterogeneous in cerebral ischemia.[7] Reperfusion does not restore this balance, which is still reduced and heterogeneous.[6] New agents that increase cell survival and improve oxygen supply/consumption balance could act to reduce further the degree of damage after cerebral ischemia.[8,9]

The mammalian target of rapamycin (mTOR) is a protein kinase that promotes cell growth and survival that might prove to be a viable target for treatment of the damaged or

From the Rutgers Robert Wood Johnson Medical School, Neuroscience & Cell Biology, Piscataway, New Jersey.

Received January 3, 2019; revision received June 21, 2019; accepted June 26, 2019.

Financial Disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Address correspondence to Harvey R. Weiss, PhD, Rutgers Robert Wood Johnson Medical School, Neuroscience & Cell Biology, 675 Hoes Lane West, Piscataway, NJ 08854. E-mail: hweiss@rwjmsrutgers.edu.

1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.06.034

ischemic brain.[10,11] However, results have been inconsistent, with some groups showing a benefit from rapamycin treatment and others showing increased damage after cerebral ischemia.[12-14] This discrepancy may be related to the complex signaling system with two protein complexes, mTORC1 and mTORC2, that play a role in the control of cellular metabolism.[15,16] The best-known rapamycin-sensitive mTORC1 target is S6 kinase (S6K1), a protein kinase that phosphorylates the ribosomal subunit S6 to control translation initiation. S6K1 is also essential for protein synthesis, cell growth, and proliferation.[16,17] S6K1 could also have vascular effects that are related to its effects on HIF-1alpha and VEGF.[18] There are reports that inhibition of S6K1 by PF-4708671 produced significant neuroprotection during hibernation and axonal regeneration in spinal cord injury.[19,20] However, the effects of inhibition of S6K1 following cerebral ischemia-reperfusion have not been reported.

In the current study, we tested the hypothesis that inhibition of p70 ribosomal S6K1 with PF-4708671 would reduce local brain damage after cerebral ischemia-reperfusion and also improve microregional O_2 supply/consumption balance in the ischemic-reperfused region. This was tested in isoflurane-anesthetized rats subjected to middle cerebral artery (MCA) occlusion and reperfusion with and without PF-4708671 treatment. We found that S6K1 inhibition with PF-4708671 reduced the damage after cerebral ischemia-reperfusion and improved microregional oxygen balance in the injured rat brain. This study suggests that inhibition of S6K1 might prove to be a useful additional treatment to reduce damage after cerebral ischemia-reperfusion.

Material and Methods

This investigation was conducted in accordance to US Public Health Service Guidelines using the Guide for the Care of Laboratory Animals (DHHS publication no. 85-23, revised 1996) and was approved by our Institutional Animal Care and Use Committee. Sixteen male Fischer 344 rats (3 months) were randomly divided into a cerebral ischemic-reperfused (n = 8) and PF-4708671 treated ischemic-reperfused (n = 8) group. In the PF-4708671 (Sigma-Aldrich) treated animals, 75 mg/kg of PF-4708671 was dissolved in 20% DMSO plus 10% Tween-80 in normal saline and injected ip, 15 minutes after the onset of ischemia. In the control group, vehicle was injected. All animals completed the protocol and were used in the analysis. Each rat was used to measure regional cerebral blood flow and microscopic arterial and venous oxygen saturations.

The rats were initially anesthetized with 2% isoflurane in an air and oxygen mixture through a tracheal tube to maintain the arterial PO_2 at about 100 mm Hg. A femoral artery and vein were cannulated. The venous catheter was used to administer the radioactive tracer. The arterial catheter was connected to a pressure transducer and an Iworx data acquisition system to monitor heart rate and blood pressure. This catheter was also used to obtain arterial blood samples for analysis of hemoglobin, blood gases, and pH using a Radiometer blood gas analyzer. The isoflurane concentration was decreased to 1.4 %. Body temperature was monitored and maintained at 37°C with a servo-controlled rectal thermistor probe and a heating lamp.

We transiently occluded the MCA using an intraluminal thread to study cerebral ischemia-reperfusion.[6,21,22] The common carotid artery was exposed through a midline ventral cervical incision and carefully separated from the adjacent nerve. Then a 4.0 monofilament thread with its tip rounded was inserted into the stump of the external carotid artery and advanced approximately 1.7 cm into the internal carotid artery until resistance was felt. The filament was held in place for 60 minutes blocking the MCA and then it was removed, allowing reperfusion, and the external carotid artery was closed. Measurements were performed after 120 minutes of reperfusion. Regional cerebral blood flow and microscopic O_2 saturations of small veins and arteries were determined in 3 brain regions in both groups of animals.

Regional cerebral blood flow was measured by the ^{14}C-iodoantipyrine quantitative autoradiographic technique. Briefly, 40 μCi of ^{14}C-iodoantipyrine was infused intravenously. When the isotope entered the venous circulation, the arterial catheter was cut to 20 mm to minimize smearing. Twenty μL blood samples were obtained from the arterial catheter approximately every 3 seconds during next 60 seconds. At the moment that the last sample was obtained the animal was decapitated and the head was frozen in liquid nitrogen. While frozen, the brain was sampled from 3 regions: ischemic cortex, contralateral cortex, and pons in a blinded fashion. The brain samples were sectioned (20 μm) on a microtome-cryostat and the sections were exposed to X-ray film to obtain an autoradiogram. The cerebral ^{14}C-iodoantipyrine concentrations were determined by reference to precalibrated standards using the NIH imageJ program. For each brain region examined, a minimum of 8 optical density measurements were made, each on different sections. Blood samples were placed in a tissue solubilizer and 24 hours later put in a counting liquid. These samples were counted on a liquid scintillation counter and were quench corrected. Regional cerebral blood flows were then calculated.

Alternate sections from regions of the same brain (ischemic cortex, contralateral cortex, and pons) were used for the determination of arterial and venous O_2 saturation. The cortical regions were from a ~5 mm plug from the parietal cortex over the MCA. Details of this technique have been published previously.[7,23] Brain sections were cut into wafers at −20°C. Twenty micron thick sections were obtained at −35°C under a N_2 atmosphere. The sections were transferred to precooled glass slides and covered with degassed silicone oil and a coverslip. These slides were placed on a microspectrophotometer fitted with an N_2-flushed cold stage to obtain readings of optical density at 568, 523, and

560 nm. This 3-wavelength method corrects for the light scattering in the frozen blood. Only vessels cut in transverse section were studied so that the path of light traversed only the blood. The size of the measuring spot was 8 μm in diameter. Readings were obtained to determine O_2 saturation in 5 arteries and 10 veins (20-60 μm in diameter) in each region. The O_2 content of blood was determined by multiplying the percent O_2 saturation by the hemoglobin concentration times 1.36, the maximal binding capacity of hemoglobin for O_2 per gram. The difference between the average arterial and venous O_2 contents (regional O_2 extraction) was then obtained. Using the Fick principle, we calculated the O_2 consumption for each region as the product of average flow and O_2 extraction. This method has been validated in the brain[7].

An additional 5 rats in each group were used to determine the size of the infarct. The brain was removed and was sliced in coronal sections. There were typically 3-4 slices of approximately 2-3 mm thickness of brain tissue each. For tetrazolium staining, a .05% solution of 2,3,5-triphenyltetrazolium chloride (Sigma) solution in PBS was prepared and warmed to 37°C. The brain slices were placed in the solution and were incubated for 30 minutes. The tetrazolium chloride solution was then poured off and the slices were washed 3 times in PBS, 1 minute per wash. To keep the slices from drying out, each slice was placed in a small weighing boat with PBS. The slides were then scanned in a blinded fashion and the scanned images were measured for total and infarcted areas in the cortex using NIH ImageJ. Cortical infarcted areas were reported as percent of total cortical area.

Analysis of variance using a repeated measure design was performed for the various measurements to assess the difference between the different regions and treatments with regard to hemodynamic, blood gases, cerebral blood flow, O_2 extraction, and O_2 consumption values. Posthoc testing of multiple comparisons was performed using Tukey's procedure. The coefficient of variation (CV) of venous oxygen saturations (SvO_2) was used to compare changes in heterogeneity. The CV was calculated as 100 × SD/mean. A χ^2 test was used to assess differences in the distribution of SvO_2 and differences in the number of low O_2 saturated veins between groups. A value of $P < .05$ was considered as statistically significant. All values are expressed as mean ± S.E.M.

Results

Hemodynamic and blood gas parameters in the ischemic-reperfused (1 hourr MCA occlusion + 2 hours reperfusion) and PF-4708671 treated ischemia-reperfusion groups of rats were within the normal ranges for anesthetized rats (Table 1). There were no statistically significant differences in arterial blood pressures between groups. Heart rates were also similar between the 2 groups of rats. Arterial blood gases and pH were controlled and also were not significantly different between the 2 experimental groups.

Table 1. *Hemodynamic and blood gas values for the ischemic-reperfusion (N = 8) and PF-4708671 ischemic-reperfusion (N = 8) groups*

	Ischemic-reperfused group	PF-4708671 ischemic-reperfused group
Systolic blood pressure (mm Hg)	122 ± 10	112 ± 8
Diastolic blood pressure (mm Hg)	80 ± 7	68 ± 61
Mean blood pressure (mm Hg)	94 ± 8	84 ± 6
Heart rate (beats/min)	268 ± 20	288 ± 18
Arterial PO_2 (mm Hg)	104 ± 7	113 ± 6
Arterial PCO_2 (mm Hg)	30 ± 3	28 ± 2
pH	7.34 ± .02	7.27 ± .02

Values are mean ± SEM (N = 8 per group).

Cerebral blood flow in the ischemic-reperfused cortex was slightly, but not significantly, lower compared to the contralateral cortex in the control group, Figure 1. In the PF-4708671 group, flow in the ischemic-reperfused region was also similar to the value in the contralateral cortex. The differences in blood flow between the control and PF-4708671 groups were not statistically significant in either the ischemic-reperfused or the contralateral cortical regions

The oxygen extraction of the ischemic-reperfused cortex was significantly elevated (+35%) compared the contralateral cortex in the control group, Figure 1. Similarly, the oxygen extraction was also significantly elevated in the PF-4708671 group in the ischemic-reperfused region (+42%) compared to the contralateral cortex. There were no significant differences in O_2 extraction in comparison between the groups in the ischemic-reperfused or the contralateral cortical regions.

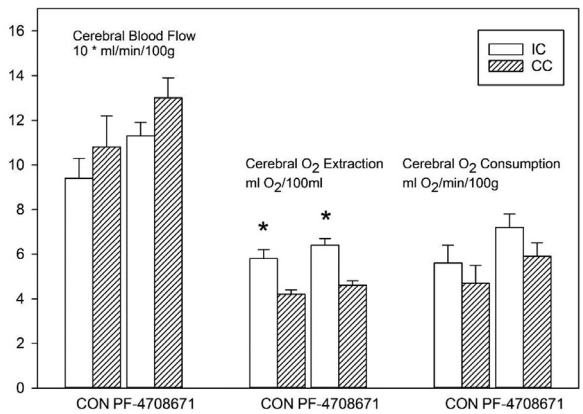

Figure 1. *Cerebral blood flow (left), O_2 extraction (center), and O_2 consumption (right) in the ischemic-reperfused cortex (IC) and contralateral cortex (CC) of the control (CON, n = 8) and PF-4708671 (PF-4708671, n = 8) groups subjected to 1 hour of MCA occlusion and 2 hours of reperfusion. * Significantly different from comparable control region.*

Cerebral O_2 consumption in the ischemic-reperfused cortex was not significantly different from the value found in the contralateral cortex in both the control and PF-4708671 treated groups of rats, Figure 1. In addition, cerebral oxygen consumption was not significantly different in examination of these cortical regions in comparison between the control group and PF-4708671 group.

Arterial O_2 saturations were similar in all regions and groups (data not shown). However, venous O_2 saturation was significantly lower in the ischemic-reperfused region compared to the contralateral cortex in both groups. The venous O_2 saturation was significantly higher in the contralateral cortex compared to the ischemic reperfused region of both the control and PF-4708671 group (control contralateral 62.3 ± .4%, control ischemic-reperfused 50.0 ± 2.1%, PF-4708671 contralateral 63.1 ± .3%, PF-4708671 ischemic-reperfused 50.0 ± .4%). There were no significant differences between groups. The distribution of small vein (20-60 μm diameter) O_2 saturations was heterogeneous in all cortical regions, Figure 2. There was a shift to lower values in the ischemic-reperfused cortex compared to the contralateral cortex of both experimental groups. The number of vessels with lowered O_2 saturations was significantly greater in the ischemic-reperfused cortex compared to the contralateral cortex of both groups. However, the number of low saturation veins (<45%) was significantly greater in the ischemic-reperfused area of the control group (23 of 80) compared to the PF-4708671 group of rats (6 of 80). The (CV = 100*SD/mean, a measure of heterogeneity) increased (14.6 verus

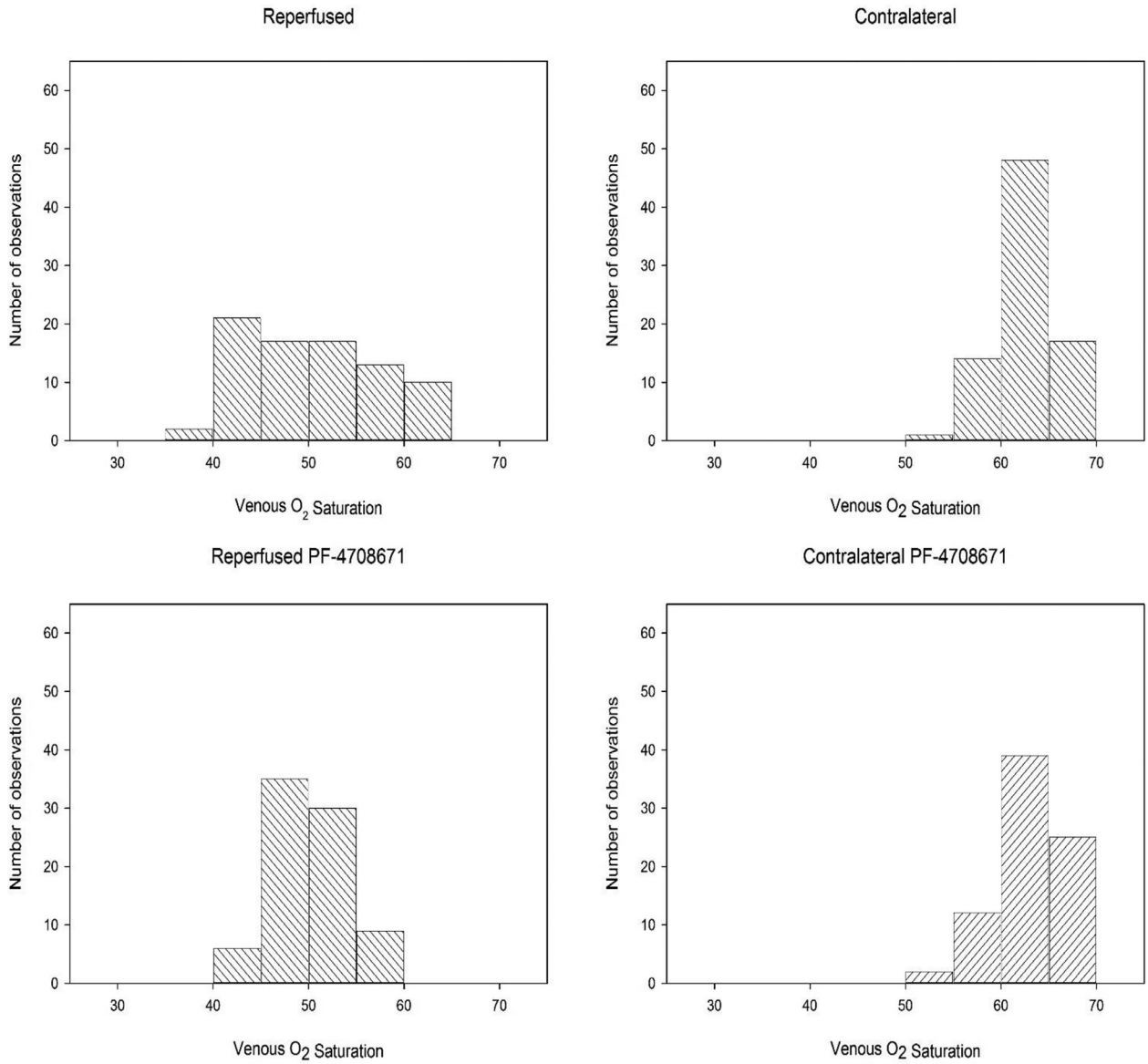

Figure 2. *Distribution of O_2 saturations (%) of small veins in the ischemic-reperfused and the contralateral cortex of the control (top, N = 80 per region) and the PF-4708671 treated group (bottom, N = 80 per region). There was a significant shift to lower values in the ischemic-reperfused cortex compared to the contralateral cortex. There were significantly less veins below 45% saturation in the ischemic-reperfused region of the PF-4708671 group compared to the control group.*

5.2) in the ischemic-reperfused cortex compared to contralateral cortex in the control group. However, the CV was similar in the ischemic-reperfused region compared to the contralateral cortex of the PF-4708671 group (7.8 verus 6.3). The ischemic-reperfused cortex of the PF-4708671 group had less low O_2 saturation veins and a narrower distribution of O_2 saturations compared to the ischemic-reperfused cortex of the control group.

The size of cortical infarct measured as the percentage of the total cortical area determined at the end of the 2-hour reperfusion period was significantly greater in the control group compared to the PF-4708671 group. The size of the cortical area of damage in the control group was 12.9 ± .8% and it was 6.6 ± .3% in the PF-4708671 group. The data for cerebral infarct size are presented in Figure 3.

We also compared the effects of PF-4708671 on brain O_2 supply/consumption parameters in a remote cerebral region, the pons. Blood flow (control: 129 ± 19 verus PF-4708671: 144 ± 14 mL/min/100 g) in the pons was not significantly different in comparison between groups. The oxygen extraction (control: 4.2 ± .1 verus PF-4708671: 4.4 ± .2 mL O_2/100 mL) was similar between the 2 groups in the pons. The oxygen consumption of the pons was also similar between groups (control: 5.4 ± .9 verus PF-4708671: 6.4 ± .8 mL O_2/min/100 g).

Discussion

Despite reperfusion of the middle cerebral artery, there was a significant area of cell death even though local oxygen consumption was restored. The average local venous O_2 saturation was significantly reduced and the number of low O_2 saturation small veins was increased in this reperfused area. This was similar to our previous reports.[6,13] The major new finding of this study was that inhibition of p70 ribosomal S6K1 with PF-4708671 reduced infarct size. In addition, the number of low O_2 saturation small veins was significantly decreased in the reperfused region demonstrating improved microregional oxygenation in the reperfused area. This suggests that inhibition of S6K1 may play a protective role after cerebral ischemia-reperfusion in this rat model.

With blockage of a cerebral artery, local blood flow and O_2 consumption are reduced.[7,24] Glucose utilization was also reported to be depressed in cerebral ischemia.[4] Cerebral oxygen extraction was also increased and venous O_2 saturations declined in the affected region. This has been observed in both cats and rats.[7,25] Tissue PO_2 was also significantly reduced.[3] Deoxyhemoglobin levels may be increased in humans with stroke.[26] Without further treatment, large parts of this area will suffer both loss of function and irreversible damage.[21,24] With reperfusion, blood flow is partially restored and O_2 consumption returns toward the value found in the contralateral cortex.[6] However, small cerebral vein O_2 saturations were significantly lowered compared to the contralateral cortex, see Figure 2. This indicated many small regions of low oxygenation within the reperfused cortical region in the control group. In addition, we found significant cell damage within the ischemic-reperfused cortex.[6] Further treatment could reduce the degree of cell death and improve local oxygenation.

The mTOR/PI3K/Akt pathway prevents apoptosis, inhibits autophagic cell death, promotes neurogenesis, and increases angiogenesis.[27] Its activation has been

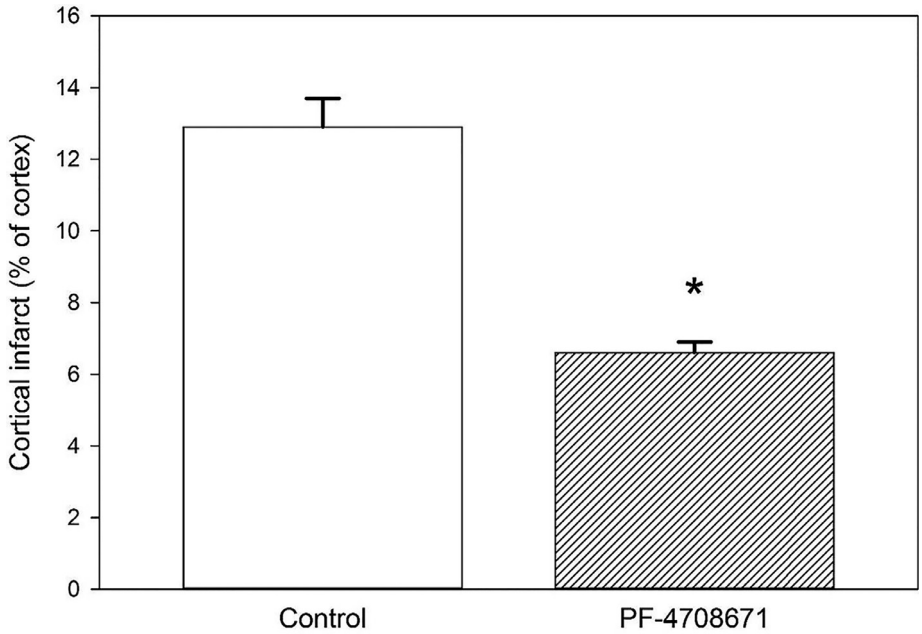

Figure 3. *Area of cortical infarction presented as percent of total cortex for the control (N = 5) and PF-4708671 (N = 5) groups. * Significantly different from control group.*

proposed to be of benefit in the treatment of ischemic stroke.[27-29] However, inhibiting mTOR with rapamycin has been associated with both positive and negative responses after cerebral ischemia-reperfusion.[13,29-31] The discrepancies are most likely due to the treatment conditions since prolonged rapamycin treatment also leads to mTORC2/Akt inhibition. Our previous study demonstrated that the effect of rapamycin was negative after cerebral ischemia-reperfusion when rapamycin also inhibited the activation of Akt.[13] In the current study, we examined the potential beneficial effects of a specific inhibition of a component of mTORC1 activation, the p70 ribosomal S6K1 with PF-4708671. We found improved oxygenation and reduced infarct size with PF-4708671.

Our data agree with other studies suggesting neuroprotection with PF-4708671 induced inhibition of S6K1. PF-4708671 increased tolerance to oxygen/glucose deprivation in an in vitro model.[20] It has also been recently reported that inhibition of S6K1 with PF-4708671 stimulated corticospinal tract regeneration after spinal cord injury with significant locomotor recovery.[19] In a study where mouse pups were subjected to hypoxia-ischemia and lipopolysaccharide-induced inflammation, PF-4708671 reduced neuronal death.[32] These studies demonstrate the important role of S6K1 in neuronal survival and regeneration following cerebral ischemia or injury. We found a significant reduction in cerebral infarct size after local ischemia-reperfusion following administration of PF-4708671 in the current study. This could be a true reduction or a delay in infarct development.

We also found that the ischemic-reperfused brain region had significantly reduced small vein O_2 saturations and increased O_2 extraction in the control group. Tissue PO_2 was reported to be significantly reduced by cerebral ischemia, but only partially restored by reperfusion.[3] Both venous O_2 saturation and tissue PO_2 are very heterogeneously distributed in the ischemic-reperfused brain with a shift to lower values compared to a control region.[3,6] Treatment with PF-4708671 reduced the number of small veins with low O_2 saturation in the ischemic-reperfused area. There was a 74% reduction in the measured veins with low oxygen saturation with PF-4708671. This provides good evidence for a restoration of local oxygen balance in the ischemic-reperfused area with S6K1 inhibition. Since cell death was also reduced, it is possible that the improved oxygenation is related to the reduced oxygen demand per cell and/or some change in the distribution of blood flow after PF-4708671.

Why some parts of the mTOR/PI3K/Akt signaling pathway are protective and others are harmful is not clear. Activating this signaling pathway has been proposed to be a potential treatment to reduce the degree of stroke damage.[27-29] We had previously shown that activating Akt was of benefit in terms of oxygen supply/consumption balance and infarct size reduction.[33] The p70 ribosomal S6K1 phosphorylates various other kinases and proteins.[34] We have demonstrated inhibition of a downstream kinase after S6K1 inhibition in the model of cerebral ischemia-reperfusion.[35] Its inhibition, may or may not be of benefit, is treatment of cancer. However, inhibition of S6K1 is associated with a reduction in age related neurodegeneration.[36] In addition, its inhibition may promote synaptic plasticity and learning.[37] These studies suggest that PF-4708671 may reduce cerebral infarct size, which is what we report. Why PF-4708671 also reduces the number of microregions in the ischemic-reperfused area is still not known. However, restricting blood flow has been reported to activate S6K1.[38] This may suggest some relationship between S6K1 and small vessel blood flow. Further work is necessary to study this mechanism.

In summary, we found a significant area of cell death even though local oxygen consumption was restored with reperfusion. The average local venous O_2 saturation was significantly reduced and the number of low saturation small veins was increased in this ischemic-reperfused area. The major new finding of this study was that blockade of S6K2 with PF-4708671 reduced infarct size and also reduced the number of low O_2 saturation small in the reperfused region. Our study suggests that blockade of S6K1 with PF-4708671 may play a protective role after cerebral ischemia-reperfusion. Further studies are necessary to determine if this protection translates to humans.

Conflict of Interest

None of the authors has any potential sources of conflict of interest or relationship, financial, or otherwise, that might be perceived as influencing the author's objectivity directly or indirectly related to this manuscript.

References

1. Alexandrov AV. Current and future recanalization strategies for acute ischemic stroke. J Intern Med 2010;267:209-219.
2. Gomis M, Davalos A. Recanalization and reperfusion therapies of acute ischemic stroke: what have we learned, what are the major research questions, and where are we headed? Front Neurol 2014;5:226.
3. Shi H, Liu KJ. Cerebral tissue oxygenation and oxidative brain injury during ischemia and reperfusion. Front Biosci 2007;12:1318-1328.
4. Martin A, Rojas S, Pareto D, et al. Depressed glucose consumption at reperfusion following brain ischemia does not correlate with mitochondrial dysfunction and development of infarction: an in vivo positron emission tomography study. Curr neurovasc Res 2009;6:82-88.
5. Tichauer KM, Elliott JT, Hadway JA, et al. Cerebral metabolic rate of oxygen and amplitude-integrated electroencephalography during early reperfusion after hypoxia-ischemia in piglets. J Appl Physiol 2009;106:1506-1512.
6. Weiss HR, Grayson J, Liu X, et al. Cerebral ischemia and reperfusion increases the heterogeneity of local oxygen supply/consumption balance. Stroke 2013;44:2553-2558.
7. Buchweitz-Milton E, Weiss HR. Effect of MCA occlusion on brain O2 supply and consumption determined microspectrophotometrically. Am J Physiol 1987;253:H454-H460. (Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.).

8. Kaur H, Prakash A, Medhi B. Drug therapy in stroke: from preclinical to clinical studies. Pharmacology 2013; 92:324-334.
9. Siket MS. Treatment of acute ischemic stroke. Emerg Med Clin North Am 2016;34:861-882.
10. Don AS, Tsang CK, Kazdoba TM, et al. Targeting mTOR as a novel therapeutic strategy for traumatic CNS injuries. Drug Discov Today 2012;17:861-868.
11. Hwang SK, Kim HH. The functions of mTOR in ischemic diseases. BMB Rep 2011;44:506-511.
12. Chi OZ, Mellender SJ, Barsoum S, et al. Effects of rapamycin pretreatment on blood-brain barrier disruption in cerebral ischemia-reperfusion. Neurosci Lett 2016;620:132-136.
13. Chi OZ, Barsoum S, Vega-Cotto NM, et al. Effects of rapamycin on cerebral oxygen supply and consumption during reperfusion after cerebral ischemia. Neuroscience 2016;316:321-327.
14. Xie L, Sun F, Wang J, et al. mTOR signaling inhibition modulates macrophage/microglia-mediated neuroinflammation and secondary injury via regulatory T cells after focal ischemia. J Immunol 2014;192:6009-6019.
15. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell 2012;149:274-293.
16. Pearce LR, Alton GR, Richter DT, et al. Characterization of PF-4708671, a novel and highly specific inhibitor of p70 ribosomal S6 kinase (S6K1). Biochem J 2010;431:245-255.
17. Tavares MR, Pavan IC, Amaral CL, et al. The S6K protein family in health and disease. Life Sci 2015;131:1-10.
18. Lee WH, Kim YW, Choi JH, et al. Oltipraz and dithiolethione congeners inhibit hypoxia-inducible factor-1alpha activity through p70 ribosomal S6 kinase-1 inhibition and H2O2-scavenging effect. Mol Cancer Ther 2009;8:2791-2802.
19. Al-Ali H, Ding Y, Slepak T, et al. The mTOR substrate S6 Kinase 1 (S6K1) is a negative regulator of axon regeneration and a potential drug target for central nervous system injury. J Neurosci 2017;37:7079-7095.
20. Miyake S, Wakita H, Bernstock JD, et al. Hypophosphorylation of ribosomal protein S6 is a molecular mechanism underlying ischemic tolerance induced by either hibernation or preconditioning. J Neurochem 2015;135:943-957.
21. Lipsanen A, Jolkkonen J. Experimental approaches to study functional recovery following cerebral ischemia. Cell Mol Life Sci 2011;68:3007-3017.
22. Sommer CJ. Ischemic stroke: experimental models and reality. Acta Neuropathol 2017;133:245-261.
23. Zhu NH, Weiss HR. Oxy- and carboxyhemoglobin saturation determination in frozen small vessels. Am J Physiol 1991;260:H626-H631.
24. Dirnagl U. Pathobiology of injury after stroke: the neurovascular unit and beyond. Ann N Y Acad Sci 2012;1268:21-25.
25. Chi OZ, Hunter C, Liu X, et al. The effects of isoflurane pretreatment on cerebral blood flow, capillary permeability, and oxygen consumption in focal cerebral ischemia in rats. Anesth Analg 2010;110:1412-1418. (Research Support, Non-U.S. Gov't).
26. Sakatani K, Murata Y, Fujiwara N, et al. Comparison of blood-oxygen-level-dependent functional magnetic resonance imaging and near-infrared spectroscopy recording during functional brain activation in patients with stroke and brain tumors. J Biomed Opt 2007;12:062110.
27. Chong ZZ, Yao Q, Li HH. The rationale of targeting mammalian target of rapamycin for ischemic stroke. Cell Signal 2013;25:1598-1607.
28. Zhao EY, Efendizade A, Cai L, et al. The role of Akt (protein kinase B) and protein kinase C in ischemia-reperfusion injury. Neurol Res 2016;38:301-308.
29. Maiese K. Cutting through the complexities of mTOR for the treatment of stroke. Curr Neurovasc Res 2014;11:177-186.
30. Crino PB. The mTOR signalling cascade: paving new roads to cure neurological disease. Nat Rev Neurol 2016;12:379-392.
31. Li Q, Zhang T, Wang J, et al. Rapamycin attenuates mitochondrial dysfunction via activation of mitophagy in experimental ischemic stroke. Biochem Biophys Res Commun 2014;444:182-188.
32. Srivastava IN, Shperdheja J, Baybis M, et al. mTOR pathway inhibition prevents neuroinflammation and neuronal death in a mouse model of cerebral palsy. Neurobiol Dis 2016;85:144-154.
33. Weiss HR, Chi OZ, Kiss GK, et al. Akt activation improves microregional oxygen supply/consumption balance after cerebral ischemia-reperfusion. Brain Res 2018;1683:48-54.
34. Ghosh J, Kapur R. Role of mTORC1-S6K1 signaling pathway in regulation of hematopoietic stem cell and acute myeloid leukemia. Exp Hematol 2017;50:13-21.
35. Chi OZ, Kiss GK, Mellender SJ, et al. Inhibition of p70 ribosomal S6 kinase 1 (S6K1) by PF-4708671 decreased infarct size in early cerebral ischemia-reperfusion with decreased BBB permeability. Eur J Pharmacol 2019;855:202-207.
36. Jahrling JB, Laberge RM. Age-related neurodegeneration prevention through mTOR inhibition: potential mechanisms and remaining questions. Curr Top Med Chem 2015;15:2139-2151.
37. Sun J, Liu Y, Tran J, et al. mTORC1-S6K1 inhibition or mTORC2 activation improves hippocampal synaptic plasticity and learning in Angelman syndrome mice. Cell Mol Life Sci 2016;73:4303-4314.
38. Fujita S, Abe T, Drummond MJ, et al. Blood flow restriction during low-intensity resistance exercise increases S6K1 phosphorylation and muscle protein synthesis. J Appl Physiol 2007;103:903-910. (1985).