Postischemic Application of Lipid Peroxidation Inhibitor U-101033E Reduces Neuronal Damage After Global Cerebral Ischemia in Rats

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Background and Purpose—The lipid peroxidation inhibitor U-101033E was examined for effects on cerebral blood flow (CBF), cortical tissue hemoglobin oxygen saturation ($HbSo_2$), and neuronal damage.

Methods—Fifteen minutes of global cerebral ischemia was induced by two-vessel occlusion and hypobaric hypotension. Wistar rats (n=25) were randomized to receive vehicle (n=9) or 40 mg/kg U-101033E (n=9) intraperitoneally during 2 hours of reperfusion. A sham group (n=7) had neither ischemia nor therapy. Histology was evaluated 7 days after ischemia.

Results—During late hyperperfusion (at 17 minutes), vehicle-treated animals had a higher ($P=0.044$) cortical tissue $HbSo_2$ (72.0±1.4%) than did U-101033E–treated animals (65.8±2.5%). Neuronal counts in the superficial cortex layer found after 7 days correlated negatively with rCBF ($r=-0.76$; $P<0.001$) or cortical tissue $HbSo_2$ ($r=-0.56$; $P=0.028$) assessed during the late hyperperfusion phase. U-101033E reduced neuronal damage in hippocampal CA1 from 64.3±9.2% to 31.2±8.4% ($P=0.020$), as well as in the superficial cortical layer from 53.5±14.6% to 12.8±11.7% ($P=0.046$). While animals in the vehicle group had reduced counts in all four examined cortex layers ($P<0.05$ versus sham group), there was significant cortical neuron loss in the U-101033E group in only one of four areas. U-101033E had no effect on resting CBF or CO_2 reactivity.

Conclusions—Postischemic application of U-101033E protects hippocampal CA1 and cortical neurons after 15 minutes of global cerebral ischemia. The results indicate that free radical–induced lipid peroxidation contributes to reperfusion injury, a process that can be inhibited by antioxidants such as U-101033E. **(*Stroke*. 1998;29:1240-1247.)**

Key Words: cerebral blood flow ■ cerebral oxygenation ■ pyrrolopyrimidine ■ U-101033E ■ vasomotor reactivity ■ rats

Free radical–induced lipid peroxidation is thought to contribute to reperfusion injury after cerebral ischemia. As soon as tissue is reperfused and hence reoxygenated, several species of oxygen-derived free radicals, such as superoxide anion radical, hydroxyl radical, and hydrogen peroxide, are generated through various pathways.[1–3] Those radicals induce lipid peroxidation, which is a chain reaction leading to alterations or destruction of cell membranes and to tissue injury.

Antioxidant compounds have been developed to attenuate neuronal damage after cerebral ischemia. The 21-aminosteroid (lazaroid) tirilazad mesylate has been demonstrated to be a potent inhibitor of lipid peroxidation and to reduce traumatic and ischemic damage in a number of experimental models.[4] Tirilazad predominantly acts at endothelial sites and has generally failed to affect delayed neuronal damage in the selectively vulnerable CA1 region as a result of its limited penetration into brain parenchyma.[5] Now a new generation of antioxidant compounds, the pyrrolopyrimidines, has been developed, with a significantly improved ability to penetrate the blood-brain barrier.[6] U-101033E, a pyrrolopyrimidine, has been shown to be a potent inhibitor of iron-dependent lipid peroxidative neuronal injury in vitro.[6] A neuroprotective effect on hippocampal CA1 neurons by repeated (preischemic and multiple postischemic) application of U-101033E in the less severe gerbil 5-minute forebrain ischemia model has been described.[6]

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The present study was designed to examine the effects of postischemic treatment with U-101033E on CBF, cortical tissue $HbSo_2$, and histological outcome after 15 minutes of global cerebral ischemia.

Materials and Methods

The present study was conducted according to the current animal protection legislation and was reviewed by the regional ethics committee (AZ 177–07/931–20).

Animal Preparation and Hypobaric Hypotension

Twenty-five male Wistar rats (250 to 360 g body wt; strain Crl:(WI) BR, Charles River) were premedicated with 0.5 mg atropine.

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Selected Abbreviations and Acronyms

CBF, lCBF, rCBF = cerebral blood flow, local CBF, regional CBF
$HbSo_2$ = hemoglobin oxygen saturation
LD = laser Doppler
MABP = mean arterial blood flow

Anesthesia was introduced with ether and continued by intraperitoneal injection of chloral hydrate (initially 360 mg/kg body wt, afterward 120 mg/kg body wt per hour). Animals were orally intubated and ventilated by a rodent respirator (Harvard) during the entire experiment. The rectal temperature was controlled at 37°C by means of a feedback-controlled homeothermic blanket control unit (Harvard, Edenbridge GB). A loose thread was looped around the left common carotid artery for temporary ligation during ischemia. A polyethylene catheter was inserted into the right common carotid artery for continuous monitoring of arterial blood pressure by a pressure transducer (Gould 134615-50) and for blood gas analysis. Pa_{O_2}, Pa_{CO_2}, and pH were determined with the use of an ABL 615 blood gas analyzer (Radiometer). Heart frequency was calculated from the arterial pressure curve by fast Fourier transformation. The peritoneum was catheterized for fluid and drug administration. The head was fixed in a stereotaxic frame (Stoelting), and the skull was exposed by a 20-mm midline sagittal skin incision. Access to the brain surface was gained through a 5×3-mm large cranial window, centered 4 mm lateral and 4 mm caudal to the bregma. During the craniotomy, the drill tip was cooled continuously with physiological saline to avoid thermal injury to the cortex. The dura was left intact (OP microscope, Zeiss). During the experiment, the skull was continuously rinsed with 37°C physiological saline, and skull temperature was measured.

The lower body portion of the animals was placed in a sealable chamber, connected to an electronically controlled vacuum pump for later induction of hypobaric hypotension.[7] To do so, the barometric pressure within the chamber could be reduced to −30 cm H_2O (−2.9 kPa), thereby causing a pooling of venous blood in the lower body portion of the rat.

Measurement of CBF and $HbSo_2$

We measured lCBF using a laser flow blood perfusion monitor (model BPM 403a, TSI) with a 0.8-mm needle probe. lCBF is expressed in LD units because the calibration of LD to absolute flow units remains controversial. The LD system has a reproducibly low biological zero,[8] and with the scanning technique described below, data from individual animals and locations may be compared.[9]

The local cortical tissue $HbSo_2$ (in percentage) was measured with the Erlangen micro–light guide spectrophotometer (EMPHO II, Bodenseewerk Gerätetechnik). The EMPHO II monitor consists of four modules: a light source, a micro–light guide, a detector, and a computer.[10,11] Parallelized light from a xenon high-pressure lamp is transmitted to the tissue surface by a central fiber surrounded by a hexagon of six detecting fibers. Light is scattered by the tissue, transmitted by these detecting fibers to a fast rotating interference band-pass filter disk (502 to 628 nm), and analyzed by a photomultiplier. The raw spectrum thus obtained is corrected on-line with the dark spectrum and with the spectrum obtained from excitation light reflected from a mirror at a set distance. The response spectrum is digitized in 2-nm increments from 502 to 628 nm, and the tissue $HbSo_2$ is calculated by an iterative best-fit procedure based on the theory of Kubelka and Munk.[12]

lCBF and $HbSo_2$ were sequentially measured at 32 (8×4) cortical locations 300 μm apart with a computer-controlled micromanipulator scanning technique. A fast analog-to-digital conversion board with on-board signal processing capacity (DAP, Microstar Laboratories) allowed us to sample data with a running average for 8 seconds (ie, approximately 10 breathing cycles) for each point of measurement. Therefore, one scan took 4 minutes.

Experimental Protocol

After a 20-minute control phase, 15 minutes of global cerebral ischemia was performed by bilateral occlusion of the common carotid arteries and MABP reduction to 42 mm Hg by hypobaric hypotension. Reperfusion was monitored for 80 minutes. Scans were performed for CBF at baseline conditions (minutes 3 and 14), ischemia (minute 5) and reperfusion (minutes 1, 7, 12, 22, 30, 45, 60, and 75) as well as for $HbSo_2$ at baseline conditions (minute 8), ischemia (minute 10), and reperfusion (minutes 17, 35, and 65).

The animals were divided into three groups. Vehicle group animals (n=9) received 10 mL/kg body wt of a vehicle solution (0.02 mol/L citric acid). U-101033E group animals (n=9) received 40 mg/kg body wt of U-101033E (Pharmacia & Upjohn Inc) in vehicle solution. Sham group animals (n=7) had neither ischemia nor therapy and were used to obtain data about neuronal density in the normal brain.

Therapy was administered intraperitoneally during reperfusion according to a blinded protocol: a bolus of 10 mg/kg body wt at minute 2, 10 mg/kg body wt per hour over 2 hours by perfusion pump, and a final bolus of 10 mg/kg body wt after 2 hours of reperfusion. Vehicle animals received identical volumes of vehicle solution.

After 7 days of survival, rats were submitted to perfusion fixation with 4% paraformaldehyde under deep anesthesia. Histological slices were prepared (3-μm coronal sections) and stained with cresyl violet. Images of respective structures were obtained with the use of a light microscope equipped with a ×10 lens (Zeiss). The images were projected onto the screen of an Amiga 2000 computer (Commodore) with a color CCD camera (SSC-C370P, Sony) and a Genlock interface (a special interface card). Standardized frames were superimposed over the video image with the use of software developed in this laboratory.[13] Dimensions of the frames were calibrated with a microscope ruler (Leitz).

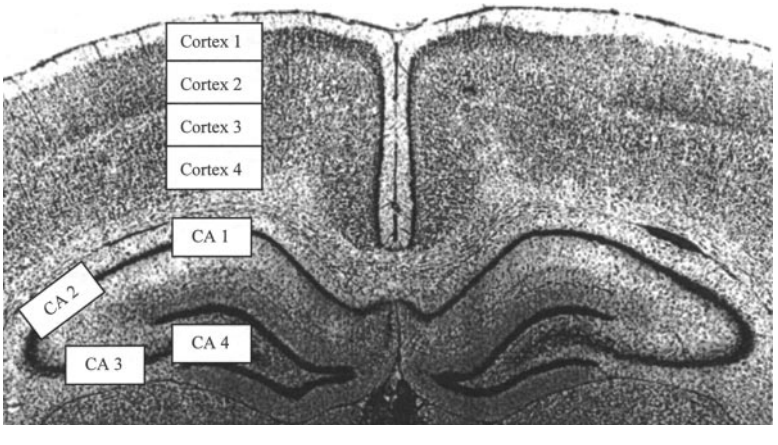

Figure 1. Location of the eight frames in a coronal section stained with cresyl violet. The frames CA1 to CA4 relate to hippocampal sectors. The four equally sized cortex 1 to 4 frames were located above the CA1 frame to achieve reproducible data. The number of intact neurons was determined inside the frames for each left and right hemisphere.

TABLE 1. MABP, Heart Frequency, Skull Temperature, and Arterial Blood Gas Analyses ($Paco_2$, Pao_2, pH) Sampled During Baseline Conditions and Reperfusion

Group	[Time]	MABP, mm Hg	Heart Frequency, min^{-1}	Skull Temperature, °C	$Paco_2$, mm Hg	Pao_2, mm Hg	pH
Vehicle	Baseline conditions	80.4±2.9	377±13	37.2±0.3	30.9±2.5	94.0±4.6	7.42±0.02
	Reperfusion min 5	83.7±4.4	365±10	36.7±0.3	28.8±2.5	107.5±4.0	7.36±0.03
	Reperfusion min 70	80.8±2.7	371±10	37.1±0.2	30.9±2.0	103.2±6.6	7.39±0.02
U-101033E	Baseline conditions	83.8±3.6	362±8	37.1±0.2	30.8±1.7	100.0±5.0	7.43±0.02
	Reperfusion min 5	82.8±3.9	362±10	37.0±0.4	29.9±1.8	110.8±4.1	7.36±0.02
	Reperfusion min 70	80.9±3.5	352±10	36.7±0.3	29.5±1.6	104.3±5.1	7.40±0.02

Values are mean±SEM. There were no statistical differences between the groups.

Frames were adjusted over the hippocampus and cortex in a defined manner, as schematically illustrated in Figure 1. The frames CA1 to CA4 relate to hippocampal areas, whereas the adjoining frames "cortex 1 to 4" were named without relation to cortex layers. The density of cortical neurons is regionally variable, and therefore the four equally sized cortex 1 to 4 frames were located above the CA1 frame (Figure 1) to achieve reproducible data. The numbers of intact neurons inside the frames were counted for the left and right hemispheres. The mean value of both hemispheres was calculated, and a neuronal damage index (100%=no vital cells, 0%=mean of sham group) was determined.

Drug Effect on Resting CBF and Vasomotor Reactivity

Additional experiments were performed to examine the effects of U-101033E on resting CBF and vasomotor reactivity in the absence of ischemia. To do so, three animals (300 to 320 g body wt) were ventilated and underwent the same surgical preparations as described above. Inspiratory and expiratory O_2 and CO_2 fractions were determined by a gas analyzer (Heyer Artema MM206C). Regional cerebrovascular CO_2 responsiveness was tested by measuring rCBF at normal and increased $PaCO_2$ values. The increase of CBF under the influence of elevated $PaCO_2$ can be best characterized by a sigmoidal curve when viewed over a large range of $PaCO_2$ changes.[14,15] Most authors, however, successfully fit a straight regression line to their experimental data when the $PaCO_2$-CBF relationship is studied in the range of 25 to 65 mm Hg $PaCO_2$. Therefore, rCBF was measured within this range at normocapnic and hypercapnic conditions, and cerebrovascular reactivity to CO_2 was calculated as the following ratio: %ΔCBF/mm Hg $PaCO_2$.[16–18]

After (normocapnic) baseline rCBF had been determined, vasomotor reactivity was studied by adding 5% CO_2 to the inspired room air.[18–20] After a steady state was achieved, rCBF was determined. Afterward, CO_2 administration was discontinued, baseline conditions were reestablished, and a control measurement was performed 5 minutes later. Then U-101033E was applied intraperitoneally (10 mg/kg body wt at minute 2, followed by continuous infusion of 10 mg/kg body wt per hour) according to the treatment protocol used in the ischemia study. rCBF measurement was performed after 15 minutes. Beginning at minute 27 during U-101033E application, vasomotor reactivity to 5% CO_2 was again determined.

Statistical Analysis

Data are expressed as mean±SEM for physiological variables and as mean±SEM of the median lCBF and $HbSO_2$ from the 32 data sets from each animal. Groups were tested for normal distribution and compared with ANOVA (Sigmastat, Jandel Scientific). Differences were assumed statistically significant at $P<0.05$.

Results

The physiological variables are summarized in Table 1. There were no statistical differences in physiological variables and skull temperature between the groups throughout the experiment.

rCBF and $HbSO_2$

During baseline conditions and ischemia there were no statistical differences in rCBF or $HbSO_2$ between the groups. Ischemia led to a decrease of rCBF from 50.6±7.9 to 3.4±0.7 LD units (vehicle group) and 41.3±4.0 to 4.3±0.8 LD units (U-101033E group); both ischemic values are close to the biological zero.

After ischemia both groups showed immediate reperfusion with postischemic hyperperfusion, which lasted for 30 minutes (Figure 2). The U-101033E group, however, reached a maximum rCBF of 66.4±5.3 LD units at 7 minutes of reperfusion and therefore earlier than the vehicle group, which showed its maximum rCBF of 67.6±4.4 LD units only at 22 minutes. Statistical differences in rCBF values were not found between the groups during reperfusion.

The vehicle group had a cortical tissue $HbSO_2$ of 64.2±2.1% compared with that of 61.3±1.3% in the U-101033E group during baseline conditions. Ischemia reduced $HbSO_2$ to 24.7±3.0% in the vehicle group and

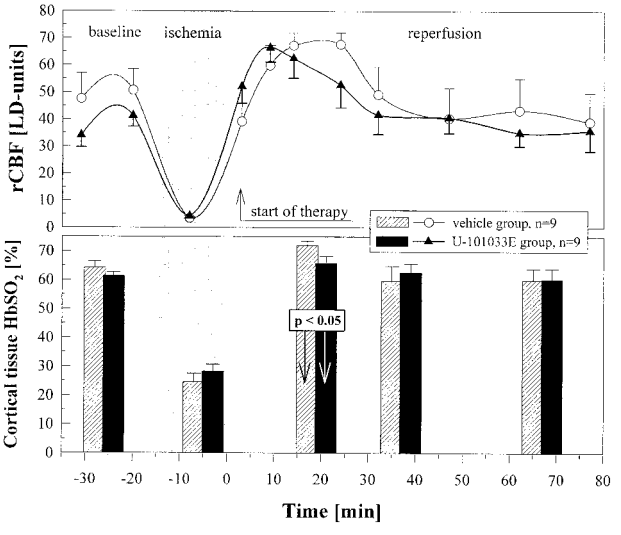

Figure 2. Regional CBF and cortical tissue $HbSO_2$ of the vehicle group (n=9, therapy with citric acid vehicle solution) and the U-101033E group (n=9, therapy with 40 mg/kg body wt in vehicle solution). Values are mean±SEM. The $HbSO_2$ differs significantly ($P=0.023$) at 17 minutes of reperfusion between the groups.

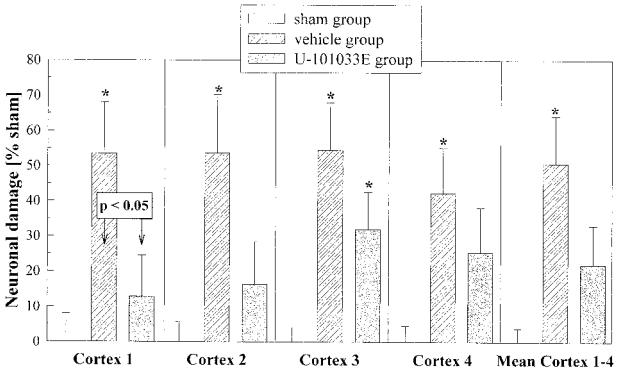

Figure 3. Neuronal damage in the cortex of the vehicle (n=7) and the U-101033E group (n=8, 40 mg/kg body wt U-101033E) 7 days after 15 minutes of global cerebral ischemia. Cortex 1 through cortex 4 are four equally sized areas (0.07 mm^2 each), with cortex 1 most superficial and cortex 4 adjacent to white matter. Neuronal damage of 0% corresponds to the mean neuronal density of the sham group (n=7) that had no ischemia. 100% damage would indicate loss of all neurons. Values are mean±SEM. *$P<0.05$ vs sham group.

28.3±2.5% in the U-101033E group. Cortical tissue $HbSo_2$ during reperfusion was equal to baseline conditions except at 17 minutes of reperfusion, when it was significantly ($P=0.023$) elevated in vehicle animals. At that time, the vehicle group had a $HbSo_2$ of 72.0±1.4%, which was significantly higher ($P=0.044$) than that in the U-101033E group (65.8±2.5%) (Figure 2).

Histological Outcome

Two vehicle-treated animals and one U-101033E–treated animal died on postischemic day 2. Treatment with the pyrrolopyrimidine significantly ($P=0.046$) reduced neuronal damage in the superficial cortical layer (cortex 1) from 53.5±14.6% in the vehicle group to 12.8±11.7% in the U-101033E group (Figure 3). Animals in the vehicle group had significant neuronal damage in all cortex layers compared with the sham group. The U-101033E group, however, had no reduced neuronal counts in the cortex, except for the cortex 3 window.

The most pronounced damage of all examined areas was found in the CA1 region, which is known to be selectively vulnerable to ischemia. U-101033E significantly ($P=0.020$) reduced the neuronal damage in this area from 64.3±9.2% in vehicle animals to 31.2±8.4% in treated animals (Figure 4). Other hippocampal areas had less damage and therefore less reduction of neuronal death by U-101033E, which was not significant.

Correlations Between Neuronal Counts and rCBF or $HbSo_2$

Neuronal counts in the superficial cortical layer significantly correlated with rCBF ($r=-0.76$; $P<0.001$) (Figure 5a) and tissue $HbSo_2$ ($r=-0.56$; $P=0.028$) (Figure 5b) measured after 22 or 17 minutes, respectively, of reperfusion. This means that a high rCBF or a high tissue $HbSo_2$, respectively, during the late hyperperfusion phase was followed by a low number of surviving neurons. At those time points, animals in the vehicle group had both an elevated rCBF and a significantly increased $HbSo_2$ compared with the U-101033E–treated animals.

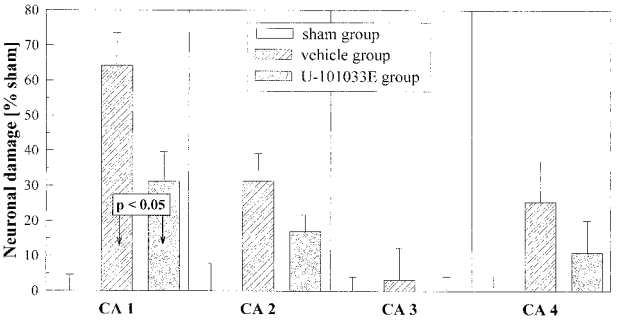

Figure 4. Neuronal damage in the hippocampus of the vehicle (n=7) and the U-101033E group (n=8, 40 mg/kg body wt) 7 days after 15 minutes of global cerebral ischemia. Neuronal damage of 0% corresponds to the mean neuronal density of the sham group (n=7) that had no ischemia. 100% damage would indicate loss of all neurons. Values are mean±SEM.

Drug Effect on Resting CBF and Vasomotor Reactivity

Application of U-101033E affected neither resting rCBF nor vasomotor reactivity to CO_2, as examined in the second study. Inspiration of 5% CO_2 significantly increased rCBF from 38.6±12.2 to 64.7±13.7 LD units ($P=0.014$) before therapy and from 37.4±9.0 to 70.1±13.5 LD units ($P=0.041$) during therapy, as shown in Table 2. CO_2 reactivity was 4.6±1.5% ΔCBF/mm Hg $PaCO_2$ before and 4.5±1.1% ΔCBF/mm Hg $PaCO_2$ during therapy (P=NS).

Discussion

Hypobaric Hypotension

Hypobaric hypotension has been shown to be an excellent method to reduce arterial blood pressure.[7] Any desired MABP

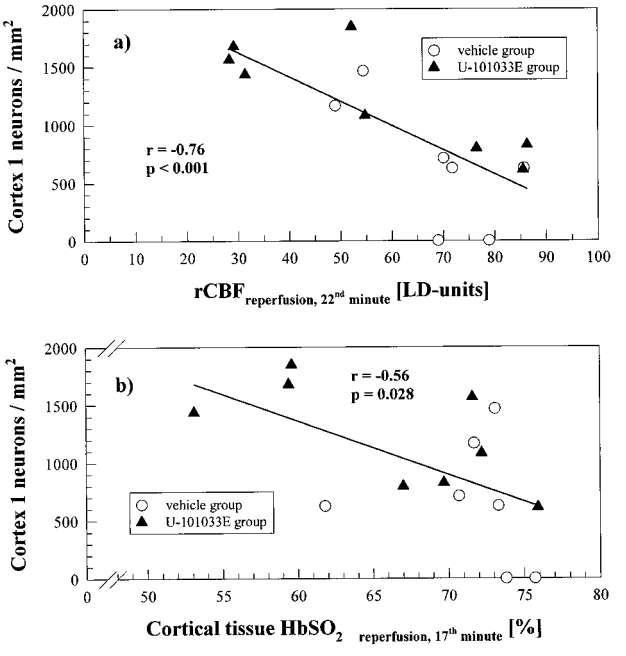

Figure 5. Correlation between neuronal counts in the superficial cortical layer and rCBF (a) or $HbSo_2$ (b) assessed during late hyperperfusion.

TABLE 2. Effects of U-101033E on Resting rCBF and Vasomotor Reactivity

	Before Therapy		U-101033E Therapy	
	Baseline	5% Inspiratory CO_2	15 min	5% Inspiratory CO_2
rCBF, LD units	38.6±12.2	64.7±13.7*	37.4±9.0	70.1±13.5†
$Paco_2$, mm Hg	40.1±0.7	59.0±2.4*	37.7±0.8	58.8±0.8†
CO_2 reactivity, %ΔCBF/mm Hg $Paco_2$	4.6±1.5		4.5±1.1	

Values are mean±SEM; n=3. The effects of U-101033E on resting rCBF and vasomotor reactivity were studied by adding 5% CO_2 to the inspired room air. Data are from rCBF and arterial CO_2 sampled before and during drug application (15 minutes).

level below the physiological MABP is adjustable by tuning the electronically controlled vacuum pump.[8] MABP was reduced to 42 mm Hg, which is below the lower autoregulation threshold that has been found to be approximately 50 mm Hg in Wistar rats.[8] Combination of hypobaric hypotension and two-vessel occlusion led to a decrease of rCBF to 4 LD units, which is close to the biological zero. Therefore, temporary global ischemia may be induced without heparinization, bleeding, or additional occlusion of the vertebral arteries.

Laser Doppler Scanning

LD flowmetry allows reliable, noninvasive, and continuous recordings of CBF with a high temporal resolution.[21,22] CBF assessment by conventional single-spot LD is highly dependent on the localization of the LD probe because of its small spatial resolution (1 mm^3). The scanning technique used in the present study helps to overcome this disadvantage and allows comparison of LD data from individual animals. The accuracy of repeated scans with the use of a stepping motor-driven micromanipulator has been found to be excellent.[8,9,23] The number of measurements necessary to obtain rCBF by local LD recordings has been evaluated, revealing that sample sizes above n=25 are necessary to obtain reliable information on rCBF,[9] a number well surpassed in the present experimental paradigm. Here, the scanning technique was expanded by including $HbSo_2$ assessment from identical locations, a procedure made possible by the similar sampling volumes of both measurement techniques.

Erlangen Micro–Light Guide Spectrophotometer

The Erlangen micro–light guide spectrophotometer (EMPHO II) allows noninvasive and continuous measurement of cortical tissue $HbSo_2$. The technique has been used in various organs,[10] including the brain.[11,24,25] The $HbSo_2$ values collected by this spectrophotometer under control conditions are very well comparable to data from other authors: Nakase et al[11] found a cortical tissue $HbSo_2$ between 50% and 60% with the same technique, and Watanabe et al[26] measured $HbSo_2$ values of 50% to 70% over capillary regions using a microreflectometric system.

Histological Outcome

The results show that 40 mg/kg body wt of the pyrrolopyrimidine U-101033E can protect hippocampal CA1 and cortical neurons from reperfusion injury after 15 minutes of global cerebral ischemia. This is consistent with the presumed role of oxygen radical–induced lipid peroxidation in postischemic neuronal damage: As soon as reperfusion after cerebral ischemia begins, oxygen-derived free radicals, such as superoxide anion radical[27–29] and hydroxyl radical,[30] are generated. This is accompanied by an increase in lipid peroxidation,[31–34] which correlates with the amount of free radical generation.[35] For the 21-aminosteroid (lazaroid) tirilazad, a correlation has been demonstrated between attenuation of oxygen radical levels and/or lipid peroxidation and the neuroprotective effect.[4] The pyrrolopyrimidine U-101033E, examined in the present study, has been shown to be a potent inhibitor of iron-dependent lipid peroxidation in vitro,[6] and the aromatized analogue U-104067F has been shown to reduce lipid peroxidation and to attenuate postischemic neuronal injury in the 5-minute gerbil forebrain ischemia model (J.A. Oostveen et al, unpublished data).

In a dose-response study using the 5-minute gerbil forebrain ischemia model, Hall et al[6] reported that 5×10 mg/kg body wt U-101033E ameliorated CA1 neuronal damage if applied preischemically and 2, 24, 48, and 72 hours after ischemia. In addition, they concluded that the therapeutic window is at least 4 hours after ischemia since they had found that a dosage of 5×30 mg/kg body wt U-101033E was effective with a delayed initial dosing at 4 hours after ischemia. In our study neuronal damage in hippocampal CA1 and cortex was significantly reduced by application of only 40 mg/kg body wt during 2 hours of reperfusion without any preischemic treatment.

Pyrrolopyrimidines are effective in focal ischemia as well: U-101033E reduces infarct size in mice after permanent middle cerebral artery occlusion[5] and in rats after temporary middle cerebral artery occlusion.[36]

Correlations

In this study a high rCBF or a high cortical tissue $HbSo_2$ in the late hyperperfusion phase correlated with a low number of surviving cortical neurons 7 days after global cerebral ischemia. This is consistent with a positive correlation between reoxygenation level and severity of neuronal damage in a rat model of 20-minute global cerebral ischemia.[37] Mickel et al[38] reported that exposure of gerbils to 100% oxygen atmosphere after 15 minutes of global cerebral ischemia resulted in increased lipid peroxidation and increased mortality.

There is an extensive inverse relationship between oxygen availability and consumption, particularly during postischemic hyperperfusion: oxygen availability increases because of hyperperfusion, and oxygen consump-

tion decreases because of the initially reduced postischemic metabolism.[39] Therefore, enough oxygen is available, which is presumably accompanied by an enhanced generation of oxygen-derived free radicals and therefore increased neuronal death.

CBF and $HbSO_2$

All three animals that died showed a pathological reperfusion pattern. Two animals (one vehicle-treated animal and one U-101033E–treated animal) showed the no-reflow phenomenon,[40] which could explain the increased mortality. The third, a vehicle-treated animal, had a continuously increasing rCBF during reperfusion, whereas the MABP remained constant. This animal reached a hyperperfusion that did not cease even at the end of the 80-minute reperfusion phase, presumably because of a loss of cerebrovascular tonus regulation.

Superoxide is generated after 20 minutes of global cerebral ischemia, with a maximum between 10 and 15 minutes of reperfusion,[29] and produces reversible dilatation of cerebral arterioles.[41] This could explain why vehicle-treated animals had a higher rCBF than U-101033E–treated animals at 22 minutes of reperfusion, which was accompanied by a significantly elevated $HbSO_2$ at 17 minutes of reperfusion. This is in accord with reduced survival of cortical neurons, as shown by the negative correlation between $HbSO_2$ and neuronal counts in the cortex.

Drug Effect on Resting CBF and Vasomotor Reactivity

The CO_2 responsiveness we found by using the LD technique (4.6% ΔCBF/mm Hg $PaCO_2$) is comparable to that in data determined in rats with other techniques (5.5% with helium clearance[42] or 3.9% with mass spectroscopy[17]). We found neither resting rCBF nor CO_2 responsiveness to be affected by U-101033E. Therefore, we conclude that the differences in postischemic rCBF and $HbSO_2$ between vehicle and treated animals are more likely due to indirect mechanisms, such as scavenging of vasodilating oxygen-derived free radicals, than to a direct vascular effect of the pyrrolopyrimidine.

Conclusions

In conclusion, this is the first study to examine the effects of U-101033E on cortical blood flow and cortical tissue hemoglobin saturation in a global cerebral ischemia model, demonstrating that the lipid peroxidation inhibitor U-101033E can protect hippocampal CA1 and cortical neurons from reperfusion injury if applied postischemically. Preischemic or additional treatment on the days after ischemia is not necessary to achieve a neuroprotective effect. Our results are in agreement with the concept that free radical–induced lipid peroxidation contributes to reperfusion injury, a process that can be inhibited by antioxidant compounds such as U-101033E. During the late hyperperfusion period, a high rCBF and a high $HbSO_2$ correlate with a low number of surviving cortical neurons. This is consistent with results that high postischemic oxygenation enhances lipid peroxidation[38] and leads to increased neuronal damage.[37] Since U-101033E reduces postischemic neuronal damage in focal[6,36] as well as in global ischemia,[6] it seems to be a promising candidate for further studies of stroke therapy.

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Editorial Comment

Since it was first suggested that free radicals are involved in the mechanisms of ischemia/reperfusion injury,[1] it has become increasingly clear that reactive oxygen species (ROS) play a critical role in ischemic brain damage.[2,3] First, cerebral ischemia and reperfusion are associated with production of ROS in the postischemic brain.[4] Second, pharmacological agents that inactivate ROS or that inhibit their deleterious effects, for example, lipid peroxidation, ameliorate cerebral ischemic damage.[5] Third, transgenic mice overexpressing the superoxide scavenging enzyme superoxide dismutase (SOD) are relatively protected from the consequences of cerebral ischemia, whereas mice with deletion of the SOD gene are more susceptible to ischemic brain injury.[6,7] Therefore, ROS scavengers or inhibitors of ROS-induced lipid peroxidation would be useful in the treatment of ischemic stroke. Unfortunately, lipid peroxidation inhibitors, such as 21-aminosteroids, did not show much promise in the treatment of patients with ischemic stroke, a result that can be partly attributed to the poor brain penetration of these agents.[8] Considering the strong evidence linking ROS generation to tissue damage resulting from reperfusion, agents with good brain penetration would be of great therapeutic value in the treatment of ischemic stroke. Their use would be particularly valuable to attempt to limit reperfusion injury in patients undergoing thrombolysis.

In the accompanying article, Soehle et al demonstrate that posttreatment with U101033E, an inhibitor of lipid peroxidation that crosses the blood-brain barrier, reduces the delayed hippocampal and neocortical damage resulting from 15 minutes of global cerebral ischemia in rats. The protection was associated with a small but significant reduction in reactive hyperemia in the early reperfusion phase. Importantly, however, in intact rats U101033E did not influence resting CBF and its reactivity to hypercapnia, suggesting that direct hemodynamic effects of the drug are unlikely to contribute to the protection. While these data provide additional evidence implicating ROS in ischemic brain injury, they also suggest that U101033E may be a promising candidate for future studies in human stroke. Additional studies in which the effect of U101033E on postischemic lipid peroxidation is tested in vivo may help

to confirm the expected mechanisms of action of this agent. In addition, studies of cerebral ischemia in species phylogenetically closer to humans would provide additional evidence supporting the potential usefulness of U101033E in patients with ischemic stroke.

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