Changes of pH and Energy State in Subacute Human Ischemia Assessed by Multinuclear Magnetic Resonance Spectroscopy

Johann Philipp Zöllner; Elke Hattingen, MD; Oliver C. Singer, MD*; Ulrich Pilatus, PhD*

Background and Purpose—In vivo changes in tissue pH and energy metabolism are key to understanding stroke pathophysiology. Our goal was to study pH changes in subacute ischemic stroke and their relation to energy metabolism, which are, unlike acidosis in acute stroke, not yet well understood.

Methods—We measured tissue pH and phospholipid as well as cell energy markers, including creatine, phosphocreatine, and N-acetyl-aspartate in subacute stroke with combined 1H and 31P magnetic resonance spectroscopy. We included 19 patients with first-ever ischemic stroke (mean time after stroke, 6 days). We then compared metabolite concentrations in the ischemic tissue to contralateral (healthy) tissue using multivariate ANOVA to assess significant differences in metabolite levels between both tissue compartments.

Results—In subacute stroke, a tissue fraction with significantly increased tissue pH was observed as compared with healthy contralateral tissue (pH, 7.09 versus 7.03; P=0.002) concurrent with splitting of the pH signal with 1 peak being more alkalotic. Furthermore, only a moderate decrease of energy-rich metabolites (phosphocreatine reduced by 17%, ATP reduced by 19%) was present, whereas total creatine was reduced by 51%.

Conclusions—The finding of an alkalotic pH split in subacute ischemia is unprecedented. The pH split and only incomplete energy loss in subacute stroke suggest 2 differently viable cellular moieties, best explained by active compensatory mechanisms after acute cerebral ischemia. (Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.114.007896.)

Key Words: energy metabolism ■ lactate ■ magnetic resonance spectroscopy ■ pH

Ischemic stroke is characterized by several key metabolic changes. Among them are changes in cell energy status, membrane lipid metabolism, and tissue pH. These changes reflect processes of cell degradation, excitotoxicity, and inflammation. Commonly, an acidic milieu is assumed in acute ischemic stroke because of a switch from aerobic to anaerobic glycolysis. This results in a reduction of tissue pH by accumulation of lactate. Several magnetic resonance spectroscopy (MRS) studies have shown acidic intracellular pH values in acute ischemia.1,2 However, a reversal from acute acidosis to subacute alkalosis has been described in longitudinal studies using MRS.3,4

Acute ischemia is expected to show a near complete depletion of energy substrates in the center of the infarct as reflected in drastically reduced levels of phosphocreatine, ATP, and increased levels of inorganic phosphate (Pi). Phosphocreatine is an important energy source to regenerate ATP from ADP by creatine kinase reaction. Owing to the breakdown of energy metabolism, the vast majority of total creatine (tCr) should, therefore, be unphosphorylated creatine (uCr). N-acetyl-aspartate (NAA), an amino acid exclusively produced in neuronal mitochondria, should be greatly reduced, as severe neuronal damage is supposed to appear in infarcted tissue. These changes in cell energy status can be assumed to persist well into the subacute phase of stroke.

Combined 1H and 31P magnetic resonance spectroscopic imaging (MRSI) at 3 Tesla is able to measure important metabolites of the above-mentioned processes in vivo. We, therefore, aimed to measure the pathophysiological fate of neuronal and glial tissue in 19 first-ever stroke patients focusing on alterations of tissue pH and energy metabolism, especially concerning the as of yet incompletely described fate of ischemic tissue in the subacute stage.

Materials and Methods

Subjects
We studied 19 adult patients suffering a first-ever ischemic stroke. The diagnosis of ischemic stroke was established clinically and by standard computed tomography or MRI. Patients were studied in the subacute phase of stroke (3 days to 2 weeks after stroke onset). Patients who had previously suffered (clinically evident or silent) strokes were excluded, as were those with a positive medical history of other brain pathologies. Further exclusion criteria were cerebral microangiopathy or hemorrhagic transformation of infarcted tissue. Patients with contraindications for MRI examination were excluded.

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as well. The study was approved by the ethics committee of the medical faculty of the Goethe University, Frankfurt. Each patient signed an informed consent form before the examination.

MRS Imaging

Magnetic resonance examinations were performed on a 3.0 T whole-body scanner (Siemens TRIO, Siemens AG, Erlangen, Germany). A $^1$H/$^31$P double-tuned head-coil (Rapid Biomedical, Würzburg, Germany) was used to obtain anatomic and spectroscopic data with the same equipment (ie, no repositioning required during scanning). We acquired axially oriented T2w anatomic images (slice thickness, 5 mm) for stroke localization aligned at the anterior–posterior commissure-axis to facilitate congruence between images. At the end of the magnetic resonance examination, a 3D T1-sequence (three-dimensional magnetization-prepared rapid gradient-echo imaging) was recorded for tissue segmentation into white matter, gray matter, and cerebrospinal fluid.

For in vivo MRS, MRSI sequences were used. The $^1$H data were obtained using an axial 2D-MRSI sequence with a slice thickness of 12 mm. The volume of interest was chosen to include infarcted tissue, as well as the respective contralateral tissue, which served as control region. We used point-resolved selective spectroscopy combined with outer volume suppression, applying an acquisition-weighted circular phase-encoding scheme on a 24×24 matrix, FOV of 240×240 mm, nominal voxel size of 10×10×12 mm, repetition time=1500 ms, echo time=144 ms, and 2 acquisitions. For $^{31}$P MRS, a three-dimensional MRSI sequence with WALTZ4 proton decoupling was used. A slice slab of 100 mm aligned to the axial $^1$H MRSI slice was recorded, signal was acquired as free induction decay applying a 60° flip angle MRSI sequence with W ALTZ4 proton decoupling was used. The spectra was restricted to the metabolites NAA, lactate, creatine (tCr), tCho, and alanine (Ala), but only NAA, tCr, and tCho were detected with Cramér-Rao lower bounds (SD values given by the tool) of 20%. Voxels were averaged for further processing whenever >1 voxel had been selected. Lactate was evaluated separately by examining individual voxels to distinguish a possible lipid signal overlap. If a clear negative doublet at 1.3 ppm was discernible and SD was <25%, the voxel was assumed to contain lactate.

For the $^{31}$P data evaluation, the grid was automatically shifted in the axial direction to place the $^1$H slice at the center of a $^{31}$P slice. To minimize partial volume effects from less affected tissue, an in-plane grid-shifting was applied manually positioning at least one voxel in T2w-positive infarcted tissue. Only 1 voxel in the center was included in the analysis, which was performed with the software jMRUI (version 4.0; http://www.mrui.uab.es/mrui). We used the AMARES algorithm, which applies a nonlinear-least-square fit of the metabolites in the frequency domain. The algorithm can handle constraints based on previous knowledge. In our case, the fit was restricted to the metabolites ATP, phosphocholine, phosphoethanolamine, phosphocreatine, glycerophosphocholine, glycerophosphoethanolamine, and Pi. Figure 1 shows example of $^{31}$P and $^1$H magnetic resonance spectra in 1 patient with left middle cerebral artery stroke.

Tissue pH calculation was performed by 2 different approaches. After the procedure described by Levine et al.,3 the frequency area for signals from inorganic phosphate at 3.6 to 5.6 ppm relative to phosphocreatine at 0 ppm was analyzed by a single Lorentzian line with variable line width, frequency, and intensity (single-peak approach). The position of this peak was used to establish a general pH value. In all but 1 case (with the smallest infarct size) the improved spectral resolution at 3 T (compared with the study at 1.5 T from Levine et al.)3 allowed a clear distinction of non-Lorentzian signal broadening in the area between 4 and 6 ppm as it is shown in Figure 2. Assuming that the broadening is caused by an additional Pi component reflecting infarcted tissue, we analyzed the area separately (double-peak approach). For this, we used 2 signals, Pi(1) and Pi(2). Pi(1) was fixed at the position of inorganic phosphate from healthy tissue (4.82 ppm), whereas the position of Pi(2) was variable between 4.85 and 5.5 ppm. Both signals had a fixed line width of 12 Hz. Resulting signal intensities and estimated pH values for Pi(1) and Pi(2) are plotted in Figure 3 against the stroke volume. For both approaches, the tissue pH was calculated from the shift of the relative Pi peaks using the formula by Petroff et al.,2 which is implemented in jMRUI.

Stroke lesion size was calculated on the basis of T2w images using the software ImageJ (http://rsbweb.nih.gov/ij/). Every slice was evaluated for visible ischemic lesions. All T2w positive areas were quantified using the polygon selection tool and the total area was then multiplied with the slice thickness.

Segmented images of white matter, gray matter, and cerebrospinal fluid were obtained using the segmentation algorithm implemented in the Statistical Parametric Mapping (SPM)-8 (Wellcome Department of Imaging Neuroscience, University College of London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) tool as described previously.22 The relative amount of gray matter, white matter, and cerebrospinal fluid contributing to the individual voxel was calculated using MATLAB scripts.6

Figure 1. $^{31}$P and $^1$H magnetic resonance spectra from a patient with left middle cerebral artery (MCA) stroke. Main metabolite signals for $^1$H are decreased in $^1$H magnetic resonance spectroscopy but lactate is visible. The broadening of the pH peak in the ischemic area is clearly distinguishable from the narrow Pi peak in contralateral (healthy) tissue. Phosphocreatine (PCr) is reduced but still detectable in the center of the large MCA stroke. GPC indicates glycerophosphocholine; GPE, glycerophosphoethanolamine; NAA, N-acetyl-aspartate; PCho, phosphocholine; PEth, phosphoethanolamine; Pi, inorganic phosphate; tCho, total choline; and tCr, total creatine.
Calibration of MRSI spectra was performed as described by Hattingen et al. Concentrations for 1H detectable metabolites were calculated by referring to an independent measurement of a spherical phantom containing an aqueous solution of 100 mmol/L acetate as a calibration standard. A repetition time of 10 s was applied to avoid T1 saturation. For 31P data, a spherical phantom with 20 mmol/L phosphate was used (repetition time, 60 s). Calibrated signal intensities were divided by the sum of gray matter and white matter fractions in a given voxel to correct for cerebrospinal fluid. This method does not take into account B1 inhomogeneities, but additional measurements performed by our group showed that the inhomogeneities are small compared with differences between healthy and affected tissue. Therefore, we are confident that the obtained values provide a reasonable estimate for absolute concentrations in mmol/L.

uCr concentrations were calculated by the formula 
\[ [\text{uCr}] = [\text{creatinine}] - [\text{phosphocreatine}] \]

Statistical Analysis

Results were statistically evaluated using the software STATISTICA (version 7.1, StatSoft, Tulsa, OK). Multivariate ANOVA was applied to analyze significant differences between stroke and control area considering data from different regions as repetitive measurements. The different metabolite groups (31P and pH, 1H, combined data) were analyzed separately. Significance for each metabolite was tested using contrast analysis in ANOVA. For correlation analysis, the Pearson correlation coefficient was used.

Results

Patients

Clinical data of the 19 patients are summarized in Table 1. Mean age at stroke onset was 56 years (SD, 16; range, 27–82 years), 26% were female patients. Seventeen patients had a stroke within the territory of the middle cerebral artery, 2 within the posterior cerebral artery territory. Mean infarct volume was 53.9 cm³ (SD, 54.3; range, 1.3–217.8 cm³). The mean time elapsed between stroke onset and MRSI was 6 days (SD, 2.8; range, 3–12 days).

Tissue pH and Lactate Concentrations

For the pH value obtained from the signal position using the single-peak approach, we found a significantly increased pH value in the infarct core relative to healthy tissue (7.09±0.07 versus 7.03±0.02; \( P_{=0.002} \); Table 2). Using this single-peak approach, a strong correlation between lesion volume and pH increase (\( r=0.91; P<0.0001 \)) was observed, suggesting that larger strokes...
Markers of Energy Metabolism

The time between stroke onset and first MRSI had no influence on any of the evaluated metabolite concentrations including lactate. There was a strong and significant reduction of NAA concentrations in the infarcted tissue (3.65±2.85 mmol/L) compared with contralateral, healthy tissue (10.47±1.75 mmol/L; P<0.001; Table 2). The tCr concentration was also significantly reduced in infarcted tissue (4.00±2.1 versus 8.24±1.84 mmol/L; P<0.001). The concentrations of the high-energy metabolites ATP (1.40±0.46 versus 1.73±0.31 mmol/L; P=0.008) and phosphocreatine (1.59±0.55 versus 1.31±0.42 mmol/L; P=0.06) go along with more alkalotic pH values. However, the data evaluation using 2 peaks with 1 peak adjusted to the pH of control tissue (double-peak approach) showed an increase in the signal intensity of Pi(2) with increasing stroke volume, whereas the position of this peak is rather constant indicating an alkalotic pH of 7.37±0.28 independent of the stroke volume (Figure 3). Lactate was detectable within the infarcted tissue only in 8 data sets. No significant correlation was found between lesion volume and lactate signal intensity (r=0.03; P=0.92).

Markers of Phospholipid Metabolism

By combining metabolite concentrations for tCr and phosphocreatine obtained from 1H and 31P examinations, we calculated the concentration of uCr. A significantly reduced amount of uCr was apparent in ischemic tissue (0.99±1.79 versus 4.66±1.77 mmol/L; P<0.001).

There was a significant negative correlation between stroke lesion volume and NAA in ischemic tissue (r=−0.56; P=0.03), indicating that larger infarcts go along with greater NAA reductions. Stroke lesion volume was also negatively correlated with creatine (r=−0.58; P=0.02) and phosphocreatine (r=−0.63; P=0.004).

Markers of Phospholipid Metabolism

We found a significant reduction in tCho in infarcted tissue (1.42±0.63 versus 2.15±0.40 mmol/L; P=0.007; Table 2). Glycerophosphocholine, being the main component of the tCho signal, was significantly lower in infarcted tissue as well (1.26±0.27 versus 1.51±0.28 mmol/L; P=0.007). Glycerophosphoethanolamine (1.08±0.42 versus 1.31±0.38 mmol/L) and phosphoethanolamine (1.01±0.23 versus 1.13±0.30 mmol/L) were also significantly reduced (P=0.03). No change of phosphocholine concentrations was observed in infarcted versus healthy tissue (0.29±0.11 versus 0.30±0.12 mmol/L; P=0.67).

We found a significant correlation between stroke lesion volume and reduction of glycerophosphocholine (r=−0.57; P=0.01). No significant correlation was found for all other lipid metabolites and lesion volume or time to first MRSI.

Discussion

This MRSI study of patients with ischemic stroke investigated tissue pH in subacute stroke together with intermediates of the cell energy and membrane lipid cycles, as well as neuronal and glial metabolites. Its major findings are an alkalotic milieu and an only incomplete depletion of energy-rich metabolites within ischemic tissue.

Tissue pH

We found a markedly increased tissue pH in the subacute phase of stroke. Further, we could not consistently find lactate within the ischemic tissue under investigation. Both the results seem contraintuitive but may be explained the following way: we suggest that the alkalotic pH and the frequent absence of lactate is attributable to the time point of our measurements, performed on average 6 days after stroke onset ranging from day 3 to 12. Commonly, acidosis is found in acute ischemic tissue subsequent from anaerobic glycolysis and accumulation of lactate. Several studies have shown an intracellular acidic pH and lactate in acute experimental stroke. Animal and human studies, however, have shown a switch from acidosis to alkalosis during the first days after ischemia. A previous human positron–emission tomography study also supports the hypothesis of alkalosis in subacute infarcted tissue, as it shows increased intracellular pH together with reduced oxygen extraction fraction, that is, a transient luxury perfusion. This alkalosis has been explained as a result of microglial invasion, of alternated buffering mechanisms especially by the sodium–hydrogen antiporter and as a result of cerebral edema. Our first (and widely used) approach for pH assessment (single-peak approach and lactate signal intensity) were within ischemic tissue.

Lactate was detectable within the infarcted tissue only in 8 data sets. No significant correlation was found between lesion volume and lactate signal intensity (r=0.03; P=0.92).

Mean±SD

Markers of energy metabolism

NAA 3.65±2.85 10.47±1.75 <0.001

tCr 4.00±2.1 8.24±1.84 <0.001

Phosphocreatine 3.07±0.60 3.70±0.62 <0.001

Unphosphorylated creatine 0.99±1.79 4.66±1.77 <0.001

ATP 1.40±0.46 1.73±0.31 <0.001

Pi 1.59±0.55 1.31±0.42 0.06

Lactate 2.22±2.09 0.97±0.77 0.04

Mean±SD

Markers of phospholipid metabolism

tCho 1.42±0.63 2.15±0.40 0.007

Glycerophosphocholine 1.26±0.27 1.51±0.28 0.002

Glycerophosphoethanolamine 1.08±0.42 1.31±0.38 0.03

Phosphoethanolamine 1.01±0.23 1.13±0.30 0.03

Phosphocholine 0.29±0.11 0.30±0.12 0.67

Values are given as mmol/L. NAA indicates N-acetyl-aspartate; Pi, inorganic phosphate; tCho, total choline; and tCr, total creatine.

4. Table 2. Quantitative Measurements of pH and Markers of Energy- and Phospholipid-Metabolism Within Infarcted and Healthy Tissue

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approach) suggests an increasing pH with increasing stroke size. However, the more sophisticated data evaluation using 2 distinct Pi signals gives room for additional explanations: One is that Pi(2) is actually present in all infarcts but is only visible in larger strokes because of the physical limitations of MRSI. The real pH in subacute ischemia would thus be closer to the pH derived from Pi(2) in large strokes, namely ≈7.37. The lower pH values in older single-peak studies being closer to the 7.09 value of our single-peak approach would thus be the result of averaging Pi(1) and Pi(2), with Pi(1) also resulting from partial volume effects and this method may underestimate changes in tissue pH. Therefore, we suggest that the more sensitive double-peak approach used here should be incorporated in further studies evaluating changes of the tissue pH in the time course after stroke.

We further suggest that the 2 distinct Pi signals are a key finding in interpreting the paradoxical switch of pH from acute to subacute stroke. Splitting of the pH suggests an active process in a subgroup of cells because passive adjustments would be on a continuum from acidic to pH neutral. We propose that this switch could be because of upregulated counteracting mechanisms in ischemic tissue. The hypoxia-inducible factor 1 (HIF-1) has been shown to act on important mediators of intracellular pH, such as the sodium–hydrogen antiporter and the carboxyhydrate enzyme. In brain tumors, these mechanisms have been shown to increase the pH, despite a hypoxic milieu. The more alkalotic milieu may be because of upregulated buffering processes within still viable cells. As an example, the sodium–hydrogen antiporter may be activated by inflammatory cytokines to be continuously active above a physiological threshold of tissue pH ≈ 7.05 in brain tissue.15

This hypothesis presupposes that there is still a viable amount of cells in the subacute stage of stroke that can counteract acidosis from hypoxia and the 2 Pi peaks may represent 2 different cellular entities, either neuronal and glial cells or neuronal cell populations being differentially affected by ischemia.

Our results suggest that further attention should be directed toward the role of tissue pH in the poststroke microenvironment, especially with regard to the effect on tissue transformation through active enzymatic cascades. Results by Zhao et al.16 demonstrated that the inhibition of the matrix metalloproteinase 9 yields drastically different results depending on the time of modification, being beneficial in the acute and deleterious in the subacute phase of stroke. Given the fact that enzymes of the metalloproteinase group are pH-dependent and have been shown to be activated in tissue acidosis it is of importance to study the effect of temporal changes of tissue pH on their enzymatic activity. As a consequence, further animal studies should combine histological and in vivo pH imaging methods to clarify these interactions.

We found neither a significant correlation between the existence of lactate and elapsed time to first measurement nor with the infarct size and suggest that accumulated lactate had been removed from the ischemic tissue at the time of our measurements by diffusion or partial reperfusion.

Energy Metabolism
The hypothesis of still viable cells within the infarcted tissue is further reflected in our results concerning cell energy metabolism. After breakdown of aerobic glycolysis, a pronounced reduction of energy carriers in ischemia may be expected because anaerobic glycolysis produces only 2 ATP molecules compared with the additional 36 ATP molecules resulting from oxidative phosphorylation. Surprisingly, the levels of phosphocreatine and ATP were only reduced by about one fifth (and not completely depleted). One possible explanation is in line with the apparent pH split, suggesting that a still functioning moiety of cells would contribute to the phosphocreatine and ATP signals. It is well known that the vulnerability of cerebral tissue is different between cell types and even between neurons themselves.18 We found a significant reduction in NAA, tCr, and tCho in subacute stroke; the NAA reduction to 35% of healthy levels was more prominent than that of tCr (to 49%). The predominant reduction in NAA hints at a higher susceptibility of neurons to ischemia because NAA synthesis is almost only performed in neuronal mitochondria. Creatine, however, being synthesized in neurons and especially in glial cells which are more resistant to ischemia,19 was less reduced. We found that the amount of uCr was significantly higher in healthy compared with infarcted tissue supporting the concept of a reduced energy demand within ischemic tissue. In other words, most of the remaining creatine in infarcted tissue was in fact energy-rich phosphocreatine. To conclude, different vulnerability to hypoxia, different perfusion states in the subacute phase of stroke and decreased energy demand may explain the relative maintenance of high-energy substrates within ischemic tissue.

An important limitation of our study is the relatively heterogeneous patient collective with some patients having smaller infarctions than others. Some metabolic disturbances may thus be underestimated because of partial volume effects. A major limitation is the lack of longitudinal data examining the transition from the acute to the subacute stroke phase. Perfusion-weighted imaging was not acquired at the time of scanning, prohibiting the assessment of interactions between reperfusion status and brain pH. Strengths of our study are the combined use of 1H and 31P spectroscopic and anatomic T2w imaging ensuring an excellent overlap among modalities. The combined use of 3 Tesla MRSI allowed a sophisticated analysis of tissue pH and to calculate further metabolites, such as uCr.

In conclusion, we could show that tissue pH in subacute human cerebral ischemia is characterized by a shift toward a more alkalotic milieu. This is best explained by active compensatory mechanisms because of still viable cell populations within the ischemic tissue. Further studies are necessary to clarify the effect of tissue pH on tissue regeneration after stroke.

Disclosures
None.

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Abstract

Background and Purpose: In vivo changes in the pH and energy state in brain ischemia are important. This study aimed to assess pH changes and ATP levels in subacute human ischemia using multinuclear magnetic resonance spectroscopy.

Methods: ¹H MRS and ³¹P MRS were used to assess pH and energy state in ischemic brain tissue. ANOVA was used to compare the results.

Results: In the subacute phase, pH changes in ischemic brain tissue were significant. ATP levels were lower compared to normal brain tissue.

Conclusion: Multinuclear MRS is a useful tool for assessing pH and energy state in subacute human ischemia.