Persistent high lactate level as a sensitive MR spectroscopy indicator of completed infarction

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Serial proton (1H) and phosphorus-31 (31P) magnetic resonance (MR) spectroscopy of cerebral infarction was performed in rats to assess the sensitivity of these techniques for use in clinical cerebral infarction. In this experimental chronic infarction model, 31P spectroscopy tended to return to a "normal" pattern within 24 hours after induction of infarction in spite of pathologically proven completed infarction and, therefore, appeared not to be sensitive enough for clinical application. On the other hand, proton spectroscopy invariably showed persistent high lactate levels and was capable of distinguishing completed infarction from reperfused recovered brain. Persistent high lactate levels appear to be a good MR spectroscopic indicator of completed infarction.

KEY WORDS • lactate • cerebral infarction • magnetic resonance spectroscopy • rat

STUDIES of dynamic cellular energetics by 31P magnetic resonance (MR) spectroscopy in vivo have provided much critical information regarding brain metabolism under ischemic conditions in experimental animals. Subsequent application of 31P MR spectroscopy to human infarction, however, was rather disappointing. Indeed, the 31P spectral pattern of the infarcted brain in the subacute or chronic phases is reported to be virtually indistinguishable from the spectra of normal brain. These findings have been attributed to the heterogeneity of the cell population observed by MR spectroscopy. Contamination of signals by surviving or migrating live cells is thought to be responsible for production of the apparently normal pattern in subacute or chronic infarction. Inorganic phosphate (Pi), a good indicator of ischemia, is thought to be washed out from cells quickly, and therefore cannot be used as a probe of nonviable cells. The initial high expectation of the utility of 31P spectroscopy in the diagnosis and management of cerebral infarction, therefore, subsided.

Water-suppressed proton (1H) spectroscopy represents another powerful tool now applicable to humans. Anaerobic glycolysis and the resultant high cellular lactate level is another fundamental cell response to ischemia. Dynamic changes in cellular lactate levels can be followed quantitatively by proton spectroscopy. Reperfusion of ischemic cells effectively reverses the cellular lactate accumulation initiated by ischemia. If lactate remains in ischemic and/or dead cells without being washed out as quickly as Pi, lactate can serve as a sensitive indicator of the "persistence" or "irreversibility" of the ischemic effects, or "completed infarction." In this study, we investigated the longitudinal changes in cellular lactate levels in cerebral infarction using an experimental infarction model in rats.

Materials and Methods

Chronic Infarction Model

Sprague-Dawley rats, each weighing 350 to 400 gm, were used. Middle cerebral artery occlusion was created under pentobarbital anesthesia in an aseptic fashion using the method of Chen, et al., with minor modifications as follows. A small craniotomy (2 × 2 mm) was made 1 mm rostral to the anterior junction of the zygomatic and squamous bones. The right middle cerebral artery was exposed by a small incision in the dura and ligated with 9-0 nylon suture. The craniotomy was closed using Gelfoam and the overlying skin was sutured. Subsequently, the common carotid arteries were exposed from a ventral midline incision and the left
common carotid artery was ligated. Acute infarction was induced by temporarily occluding the right common carotid artery with a nontraumatic aneurysmal clip for 1 hour.

**Magnetic Resonance Spectroscopy**

A 4.7-tesla system was used for MR spectroscopy.* A round surface coil (two turns, 6 mm diameter) was constructed from No. 14 copper wire with Teflon insulation tunable to the resonance frequency of proton and $^{31}$P. The animals were anesthetized with pentobarbital, 50 mg/kg, and placed in the probe. The scalp and temporal muscle were retracted to avoid signal contamination. For each animal, spectra were obtained from the intact hemisphere (control side) as well as from the infarcted hemisphere (experimental side) by placing the surface coil over the appropriate side of the calvaria. Field homogeneity was maximized by shimming on water proton signals. Proton spectroscopy was performed using a 133T-2662 sequence with $\tau$ delay of 68 msec. The interpulse delay of the Hore sequence was adjusted such that the lactate resonance would produce the least attenuation (recycle time 1.5 seconds). $^{31}$P spectra were obtained at 80.99 MHz using a one-pulse sequence with a spectral width of 6 K into a 4-K memory block (recycle time 1.6 seconds). The saturation factor for the $^{31}$P resonances was not determined. Chemical shifts of the proton and $^{31}$P spectra were referred to an external tetramethylsilane and internal phosphocreatine (PCr) resonance, respectively. The remaining free induction decay-like water signal in the proton spectra was suppressed by applying sine function apodization. The broad resonance with short $T_2$ in the $^{31}$P spectra was removed using the convolution difference technique. The relative areas of resonances were determined with a standard deconvolution program (Nicolet NMCCAP). The animals were studied at 1 hour, 24 hours, and 1 month after induction of infarction.

**Pathological Examination**

The animals were sacrificed with an overdose of pentobarbital and the brains were removed and fixed.
Persistent high lactate level in completed infarction

Fig. 3. Photomicrographs of infarcted brain 24 hours (left) and 1 month (right) after induction of infarction. There is a large number of ischemic cells in the 24-hour infarction, while the 1-month infarction shows gliosis.

in 10% formalin. Brain sections were prepared and stained with hematoxylin and eosin, and examined microscopically.

Results

Figures 1 and 2 show representative $^3$P and proton spectra, respectively, of the normal hemisphere (a) and of the infarcted hemisphere at three different stages of infarction: 1 hour (b), 24 hours (c), and 1 month (d) after induction of infarction. One hour after induction of the infarction, an increase in Pi and a decrease in PCr in the $^3$P spectrum were apparent (Fig. 1b). Within 24 hours, the increase in Pi subsided and the $^3$P spectrum became almost indistinguishable from normal brain, although the decrease in PCr appeared to persist (Fig. 2c). On the other hand, the increase in the lactate level shown in the proton spectra clearly persisted 24 hours after induction of the infarction (Fig. 2). Table 1 summarizes the data from eight animals at each of the three stages of infarction. The relative lactate level was expressed as lactate/N-acetyl-aspartate. The estimated lactate concentration 24 hours after induction of infarction was approximately 5 mM.

![Image](https://example.com/image.png)

**TABLE 1**

*Lactate/N-acetyl-aspartate levels in various brain samples*

<table>
<thead>
<tr>
<th>Brain Sample</th>
<th>Lactate Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal side</td>
<td>not detectable</td>
</tr>
<tr>
<td>experimental side</td>
<td></td>
</tr>
<tr>
<td>1 hour</td>
<td>2.52 ± 0.4†</td>
</tr>
<tr>
<td>24 hours</td>
<td>0.81 ± 0.3†</td>
</tr>
<tr>
<td>1 month</td>
<td>not detectable</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for eight samples each. The experimental side results are given for three different times after induction of infarction.
† Significance of difference: p < 0.001 by t-test analysis.

Figure 3 shows two representative histological sections 24 hours (left) and 1 month (right) after the infarction. While the 24-hour infarction shows a large number of cells with ischemic changes, the 1-month infarction shows gliosis.

Discussion

The ability to monitor cellular energetics and other metabolic parameters of cells continuously under acute ischemic conditions is of fundamental importance to biological research as well as to clinical medicine. While $^3$P MR spectroscopy has proven to be a useful tool in experimental ischemia, its application to clinical infarction has been disappointing, primarily because it fails to distinguish between reperfused recovered infarction and subacute completed infarction. The current study indicated that lactate assay by proton spectroscopy is a sensitive method for assessing cellular conditions during the subacute phase of cerebral infarction and possesses a strong potential to be a useful clinical tool.

The brain is highly dependent on glucose for its energy production. Following acute ischemia, brain tissue quickly accumulates lactate as a result of anaerobic glycolysis. If the brain tissue is reperfused and remains viable, the cellular lactic acidosis reverses. Intracellular pH and cellular lactate accumulation are inversely correlated. However, intracellular pH as measured by $^3$P spectroscopy also tends to return to normal or even rebounds into the alkalotic range during the subacute phase of infarction in the presence of histologically proven completed infarction. Therefore, $^3$P spectroscopy cannot be used as a reliable indicator for assessing completed as opposed to reperfused infarction. On the other hand, as shown in the present study, lactate remains in high concentrations during the subacute phase of infarction. The presence of lactate appears to be a sensitive indicator of completed infarction.
Quantitative monitoring of lactate levels by proton spectroscopy may provide useful information regarding the cellular metabolic status, including its response to therapeutic manipulation.

Regional lactate concentration assay is now becoming feasible with the use of MR imaging and/or localized spectroscopic techniques. This indicates that entirely new management strategies of cerebral infarction are possible in the near future. With high-field MR imaging such as with a 4-tesla system, it is possible to monitor regional lactate concentrations with good time resolution in the brain of patients with acute cerebral ischemia. With this technique combined with conventional proton imaging, management and therapeutic intervention of cerebral ischemia can be readily monitored by regional lactate assay.

References


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