Early In Vivo MR Spectroscopy Findings in Organophosphate-Induced Brain Damage—Potential Biomarkers for Short-Term Survival

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Organophosphates are highly toxic substances, which cause severe brain damage. The hallmark of the brain injury is major convulsions. The goal of this study was to assess the spatial and temporal MR changes in the brain of paraoxon intoxicated rats. T2-weighted MRI and 1H-MR-spectroscopy were conducted before intoxication, 3 h, 24 h, and 8 days postintoxication. T2 prolongation mainly in the thalami and cortex was evident as early as 3 h after intoxication (4–6% increase in T2 values, P < 0.05). On spectroscopy, N-acetyl aspartate (NAA)/creatine and NAA/choline levels significantly decreased 3 h postintoxication (>20% decrease, P < 0.005), and 3 h lactate peak was evident in all intoxicated animals. On the 8th day, although very little T2 changes were evident, NAA/creatine and choline/creatine were significantly decreased (>15%, P < 0.05). Animals who succumbed had extensive cortical edema, significant higher lactate levels and a significant decrease in NAA/creatine and NAA/choline levels compared to animals which survived the experiment. Organophosphates-induced brain damage is obvious on MR data already 3 h postintoxication. In vivo spectroscopic changes are more sensitive for assessing long-term injury than T2-weighted MR imaging. Early spectroscopic findings might be used as biomarkers for the severity of the intoxication and might predict early survival. Magn Reson Med 68:1390–1398, 2012. © 2012 Wiley Periodicals, Inc.

Key words: paraoxon; imaging; status-epilepticus; lactate

Organophosphates (OPs) are toxic compounds that are used both as pesticides and as chemical warfare agents. They irreversibly inhibit acetylcholine esterase, hence, leading to a synaptic cholinergic crisis. The main cause of death as a result of OP poisoning is respiratory failure because of a combination of bronchoconstriction, respiratory muscle paralysis, and damage to the brain respiratory centers (1). As most OPs are lipophilic, they penetrate the blood brain barrier, resulting in severe brain damage. Central nervous system effects include nonspecific symptoms as irritability, restlessness, disorientation, and confusion (2). The hallmark of brain injury in severe intoxication is loss of consciousness and major convulsions up to status epilepticus (SE).

Glutamate excitotoxicity is considered the main contributor to OP-induced seizure maintenance and neuropathology (3). Glutamate in excess induces an abnormal influx of calcium into the cells. In turn, the intracellular calcium upload overactivates intracellular catalytic enzymes that can damage cell membranes, cytoskeleton, or organelle structures. All these events eventually lead to cell death and are expected to produce important cerebral biochemical and metabolic changes (4). McDonough and Shih (3) proposed a three phase “model” for the neuropharmacological processes responsible for seizures and neuropathology produced by OP intoxication. Initiation and early expression of seizures are caused by cholinergic crisis; at this stage, no neuropathology is evident. However, if not treated promptly, a transition phase occurs during which the neuronal excitation of the seizure per se perturbs other neurotransmitter systems, i.e., excitatory amino acid levels increase reinforcing the seizure activity; mild neuropathology is occasionally observed. With prolonged epileptiform activity, the seizure enters a predominantly noncholinergic phase; major neuropathology is evident in multiple brain regions. In addition, during SE there is a large increase in cerebral glucose metabolic rate and in oxygen consumption, which leads to adenosine triphosphate depletion, to lactate accumulation, and to hypermetabolic neuronal necrosis (5–7).

OP-induced morphological brain damage is more prominent in the cortex, hippocampus, amygdala, thalamus, and in the piriform cortex (8,9). Following seizure initiation, there is a pronounced edema and swelling of astrocytes especially in perineuronal and perivascular areas (3,10). This cerebral edema has been suggested to play a significant role in soman-induced brain damage by inducing compression of cerebral vasculature and to cause hypoxic brain damage as a consequence (11). Furthermore, the swollen astrocytes, a constant feature in brain edema, may release their load of glutamate and thus amplify the excitotoxic neuronal damage (12).

Magnetic resonance imaging (MRI) is a useful and reliable noninvasive imaging modality of the brain in...
various experimental and clinical cerebral pathologies. T2-weighted MR images are particularly suited to recognize areas of increased water content in the examined tissue, i.e., parenchymal edema. T2 prolongation and reduced apparent diffusion coefficient (i.e., decreased Brownian movement of water molecules found in cytotoxic edema) were found in brain tissue of rats exposed to soman. These changes were prominent in the hippocampus, temporal and piriform cortex (11,13–15). All these imaging findings were evident 3 h after the intoxication. In another animal model of pilocarpine (a cholinergic agonist)-induced seizures, areas of restriction of diffusion and T2 prolongation were also found in cerebral cortex, in the hippocampus, and in the amygdala (16). Similarly, these techniques have been shown to enable the detection of early and minimal structural changes accompanying cerebral edema in non-OP models of experimental seizures (kainic acid) (17).

Magnetic resonance spectroscopy (MRS) is a technique that is used to measure the levels of different metabolites in a tissue. This “metabolic signature” can be used to monitor in vivo changes. The main metabolites which can be studied, inter alia, by 1H-MRS include choline-containing compounds (which are used to make cell membranes), creatine (a chemical involved in energy metabolism), N-acetyl aspartate (NAA), and lactate. NAA has the strongest signal in MRS of the rodent and human brain. NAA is thought to be present predominantly in neuronal cell and considered to be a neuronal marker. Indeed, most neurological disorders involving neuronal loss or dysfunction result in reductions in brain NAA levels including Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis, and stroke. However, it is unknown if the changes are etiological or merely secondary (18).

In the last decade, there have been only a few publications about MR characteristics of OP-induced brain damage, and to our knowledge, there are no previous studies describing spatial and temporal changes of in vivo MR spectroscopic changes of the brain of OP-intoxicated animals. This study’s objectives were to describe the spatial and temporal changes in the brain of OP intoxicated animals by MRI and MRS and to correlate between these changes and the severity of clinical intoxication, i.e., mortality.

**METHODS**

**Animals**

Adult male albino Sprague-Dawley rats, weighting 300–320 g (Harlan-biotech, Jerusalem, Israel) at the beginning of the experiment, were housed under standard laboratory conditions in plastic cages, two per cage in a controlled environment with a constant temperature of 21°C ± 2°C and 12 h light/dark cycle. Food and water were available ad libitum. Care and maintenance were in accordance with the principles described in the “guide for care and use of laboratory animals” (NRC publication, 8th edition, 2011, http://oacu.od.nih.gov/regs/guide/guide.pdf). The experiment protocol was examined and approved by the institutional committee for animal experimentation as required by local law.

**Drugs**

Paraoxon, atropine sulfate, and obidoxime were purchased from Sigma (Israel). Paraoxon was diluted in a vehicle containing 40% propylene glycol. All other drugs were diluted in normal saline.

Anesthesia was induced with ~ 4% isoflurane (Vetmarket Ltd., Petah Tikva, Israel) and maintained with 1–2% isoflurane in 95% O₂ at a flow rate of 0.3–0.5 l/h. Respiratory rate was monitored throughout the entire MR experiments and was maintained between 40 and 60 breaths/min. Body temperature was maintained by a feedback system of circulating water at 39°C.

**Study Design**

Animals (n = 14) were exposed to 1.4 LD₅₀ of paraoxon (450 μg/kg, IM; 0.5 mL/kg). One minute after intoxication, all rats were treated with atropine (3 mg/kg, IM; 0.5 mL/kg) and obidoxime (20 mg/kg, IM; 0.5 mL/kg) to induce brain damage without significant mortality. Each animal had a preintoxication brain MR examination (baseline) as well as 3 h, 24 h, and 8 days postexposure. The animals were observed continuously after the intoxication and scored for motor manifestations of seizure activity on 5, 30, 60 min and then 4.5 h (post 2nd MR scanning), 24 h and 8 days after the intoxication. The clinical scoring scale was: (0) no activity, quiet periods, (1) chewing and facial clonus, (2) mild tonic clonic seizures, and (3) severe tonic clonic seizures.

**MR Protocol**

MRI experiments were performed on a 7T/30 cm horizontal bore Bruker BioSpec (Karlsruhe, Germany) MRI scanner equipped with a BGU20 gradient system capable of producing pulse gradients of 40 Gcm⁻¹ in each of the three dimensions (x, y, and z). A body coil was used as the transmit coil, and a 15-mm quadrature coil (Bruker, Karlsruhe, Germany) dedicated for the rat brain was used as the receiving coil. The MRI examination consisted acquisition of T2 maps and MR spectroscopic imaging (MRSI) of the entire brain. The T2 maps were collected with the following parameters: 12 continuous 1.35-mm coronal slices were acquired with a field of view of 2.56 × 2.56 cm² and 256 × 128 digital resolution reconstructed to a 256 × 256 matrix, resulting in an in-plane resolution of 100 × 100 (μm)². The number of echoes was set to 16 with TR of 3000 ms and TE was between 10 and 160 ms. The total imaging time was 9.5 min for each rat.

The MRSI study was performed by a water suppressed two-dimensional (2D) PRESS MRSI scan with TR/TE of 2000/135 ms, field of view of 3.2 × 3.2 cm², matrix size of 8 × 8 zero filled to 16 × 16, and a slice thickness of 4 mm. The number of scans was 16 and the total scan time was 23 min. VAPOR water suppression scheme was used, and the slice of interest was shimmed selectively by a volume selective PRESS sequence. In the PRESS sequence, the delay was set so that the lactate peak will appear in antiphase.

**Data Analysis**

T2 maps and voxel-based analysis-T2 map analysis enabled us to compare quantitatively the T2 values of
FIG. 1. MR T2 map of a rat brain overlaid by MRSI grid. Pink areas represent mainly deep gray matter voxels (mGM), mainly thalami, and blue area represents mainly white matter voxels (corpus callosum and peri-ventricular white matter; mWM). The black highlighted area represents both mWM and mGM. Different ROIs are also shown in yellow ellipses: (1) prefrontal cortex, (2) hippocampus, (3) thalamus, (4) parieto-temporal cortex, (5) piriform cortex, and (6) hypothalamus. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the different time-points after the intoxication. After the MRI protocol was completed, the raw data were analyzed using both an in-house Matlab program tool (www.cs.tau.ac.il/~oferpas/Fistuk) and the statistical parameter mapping (SPM2) program to compare the baseline groups to the groups measured postintoxication. First, all images were rotated, cropped, and normalized (with the T2 map of one representative baseline brain as a template) using the Fistuk tool. After these procedures, we used the voxel-based analysis procedure to compare the baseline groups with the intoxicated groups. Regions that expressed a statistical difference ($P < 0.005$) after a one-way-ANOVA test between the groups were highlighted on the T2 map template. To confirm the validity of our analysis, the same procedure was performed between two baseline groups. Here, as expected, none of the pixels showed a statistical difference when $P$ was set to $0.005$.

Region of interest analysis (ROI)-T2 maps were transferred onto an external computer for data processing. Representative axial slice in level of the hippocampus and hypothalamus was selected to be analyzed for T2 values. Six ROIs were selected in both hemispheres and drawn manually on axial slice. The ROIs corresponded to parieto-temporal cortex, piriform cortex, pre-frontal cortex, hippocampus, thalamus, and hypothalamus (see Fig. 1 for the definition of the ROIs used in the analysis). ROI values were analyzed using MRlcro software. Univariate Spearman’s rank correlation was used to measure the statistical dependence between changes in ROI’s T2 values and pathological findings. This statistical analysis was done with SPSS version 14.

Analysis of MRSI results -The MRSI spectra were zero filled to 8k, multiplied by a line broadening factor of 10 Hz, then phase corrected. The spectra from all of the voxels of interest were imported to Mestre-C software. The NAA, creatine, choline, and lactate peaks were then fitted to a Lorentzian function, and the area under each peak was generated from the fitted function. Metabolite levels from two different areas in the brain were selected; voxels that represent mainly white matter (mWM, blue voxels on Fig. 1) and voxels that represent mainly gray matter (mGM) (pink voxels on Fig. 1). In addition, we computed also the metabolite levels of both white and gray matter ROIs together. The NAA/creatine, NAA/choline, and choline/creatine ratios in each time point were compared to baseline ratios using Student $T$-test.

Histological Evaluation

Animals were sacrificed by decapitation 10 days following Paraoxon exposure. The brain was rapidly removed from the skull, immersed in 4% neutral buffered paraformaldehyde (pH = 7.0) at 4°C, and processed routinely for paraffin embedding. Serial, 7-μm-thick sections were cut in the coronal plane and dried in 37°C over night. Selected sections were stained with hematoxylin and eosin (H&E) for general morphology and were evaluated for neuronal damage (edema, hyperchromatic nuclei, vacuolar necrosis, and loss of cells). The characteristic brain damage following OP exposure usually includes lesions in several areas: hippocampus, piriform cortex, parietal cortex, thalamus, and hypothalamus. Overall brain damage was semiquantitatively analyzed using the following histological score: (0) intact brain, (1) enlarged ventricles + minor changes, (2) severe damage in one of the characteristic areas, (3) medium damage or damage in two different areas, (4) severe damage in all characteristic areas not including the hippocampus region, and (5) severe damage in all characteristic areas. Additional pathological scale was used for imaging-histology correlation. Brain lesions in specific areas corresponding to imaging ROIs (see Fig. 1) were semiquantitatively analyzed using the following histological score: (0) intact brain area, (1) minor changes, (2) minor changes+ few necrotic cells, (3) medium damage, (4) severe damage in part of the area, and (5) widespread severe damage in entire area.

RESULTS

Animals and Intoxication

Generalized convulsive activity (clinical grade of 2 to 3) was recorded 5 min postintoxication in 12/14 animals. Two animals developed mild convulsions (clinical grade 1). The latency to the development of generalized seizures and the duration of the SE were similar between all rats (2–3 min postintoxication). All convulsive movements disappeared, while the first anesthesia was induced (for the 3 h MR scanning; i.e., the rats awake from the anesthesia after ~ 4.5 h postintoxication without any signs of convulsions). The clinical score stayed normal till the end of the experiment (8 days). Overall survival rate was 9/14 at the end of the study. Four animals died 12–24 h after the intoxication, whereas one died after 8th day imaging.

MR Finding

When comparing different ROIs measurements, all brain areas showed increased T2 signal as early as 3 h after
intoxication compared to the baseline exam ($P < 0.05$). The maximal changes were shown in the parieto-temporal cortex (6% ± 3%), piriform cortex (5% ± 3%), and in the thalami (4% ± 3%). The severity of the imaging findings varied among intoxicated animals and although 24 h after the intoxication the only ROIs which showed significant T2 increase were in the piriform and parieto-temporal cortex ($P < 0.05$, shown in Fig. 2a), 5/14 animals showed diffuse global cortical edema (see Fig. 2b). Voxel based analysis of the T2 values in the different time points after intoxication compared to baseline levels found significant T2 prolongation in the cortex and the subcortical gray matter as early as 3 h after the intoxication (Fig. 3a). Twenty-four hours postintoxication, the T2 prolongation was prominent mainly in the piriform cortex and to a lesser degree in the thalami (Fig. 3b). As time elapsed from the intoxication, the abnormalities diminished and very little significant T2 changes were evident 8 days after the intoxication when compared with baseline levels (Fig. 3c). T2 prolongation in the animals which died during the 1st day (i.e., 12–24 h) postintoxication (4/14 animals) was significantly more diffuse and involved larger cortical and deep gray matter regions when compared to the animals which survived the entire experiment (10/14 animals survived 8 days), as demonstrated in Fig. 4.

MR spectroscopic findings (Fig. 5). The ratios of NAA to creatine and choline as well as the choline to creatine ratio were calculated for each voxel and averaged for three areas of the brain, as shown in Fig. 1. Table 1 shows the average NAA/creatine, NAA/choline, and choline/creatine ratios.
values, for all three brain areas in different time points. NAA/creatine significantly decreased as early as 3 h after the intoxication and although it increased after 24 h, it was significantly low 8 days postintoxication when compared with baseline levels. NAA/choline decreased 3 h after the intoxication and reached the lowest values 24 h after intoxication. Decreased NAA/choline level was mainly in the gray matter rich ROIs and to a lesser degree in the white matter rich ROIs. After 8 days, NAA/choline level returned to baseline levels. Twenty-four hour after intoxication, there was a trend of increased choline/creatine ratio (significant only in white matter rich voxels). A significant choline/creatine decrease was evident on the 8th day postintoxication. In animals which did not survive the intoxication (deceased during the experiment), both 3 h NAA/creatine and NAA/choline ratios were significantly lower compared to animals which survived the experiment (NAA/creatine: \( P < 0.01 \) for all brain areas and NAA/choline: \( P < 0.05 \) for mGM and mGM+mWM).

Lactate was not detected in the MRSI at the baseline preintoxication. A peak of lactate was found 3 h after the intoxication in all animals (mean lactate/creatine was 0.33 ± 0.14, see Table 1). Lactate levels returned to baseline level 24 h after the intoxication. In animals which died during the experiments, higher levels of lactate/creatine were recorded 3 h after the intoxication (0.43 ± 0.16) compared to animals which survived the experiment (0.27 ± 0.1, \( P < 0.03 \)). The lactate/creatine levels as measured 3 h postintoxication were in good positive correlation with 24 h brain edema as measured by average total T2 values (\( R^2 = 0.87 \)). This correlation was evident although three out of the 14 animals, with the highest lactate/creatine levels, died before the 24 h T2 data could be collected from them.

Histology

Ten days following paraoxon intoxication, brain lesions of varying degrees of severity were noted in all animals. Six out of surviving nine animals, whose brain was sent to histology studies, had pathological score of 3–5. The most affected areas were the hippocampus, piriform cortex, parietal cortex, and dorso-lateral thalamus. The hippocampal pyramidal cells of CA1 and CA2 demonstrated
extensive cell damage, characterized by hyperchromatic nuclei (compare Fig. 6B,C with Fig. 6B",C"). Necrotic cells were also observed in CA3 region (compare Fig. 6D with Fig. 6D"). Damaged cells were also noted in the piriform cortex, where laminar degeneration of neurons and edema were present (compare Fig. 6E,F with Fig. 6E",F"). A medium damage was detected in the parietal cortex (compare Fig. 6I,J with Fig. 6I",J"). The lesions in the thalamus were characterized mainly by vacuolar necrosis in the dorso-lateral nuclei (compare Fig. 6G,H with Fig. 6G",H").

### Imaging-Histology Correlation

No correlation was found between 3 h T2 values on different ROIs and the pathological damage in the corresponding areas in the surviving animals. A correlation was found between the T2 values 8 days after the intoxication, as read in piriform, parieto-temporal, and prefrontal cortices ROIs and the pathological damage in corresponding cortices (Spearman correlation coefficient of 0.68, 0.76, 0.76, respectively with significance of <0.05).

Average NAA/creatine, NAA/choline, choline/creatine, and lactate/creatine levels in all time-points pre-intoxication and post-intoxication are presented as mean value ± standard deviation.

mWM, voxels that represent mainly white matter; mGM, voxels that represent mainly gray matter; NAA, N-acetylaspartate.

*P < 0.05, **P < 0.005 (baseline vs. each of the time points values).

### MR Spectroscopy in Organophosphate-Induced Brain Damage

**Table 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Area</th>
<th>NAA/Creatine</th>
<th>NAA/Choline</th>
<th>Choline/Creatine</th>
<th>Lactate/Creatine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>mWM</td>
<td>1.32 ± 0.18</td>
<td>1.26 ± 0.22</td>
<td>1.07 ± 0.15</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>mGM</td>
<td>1.25 ± 0.10</td>
<td>1.13 ± 0.17</td>
<td>1.13 ± 0.17</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.28 ± 0.11</td>
<td>1.17 ± 0.18</td>
<td>1.11 ± 0.15</td>
<td>–</td>
</tr>
<tr>
<td>3 h</td>
<td>mWM</td>
<td>0.92 ± 0.92**</td>
<td>1.00 ± 0.18**</td>
<td>0.94 ± 0.14*</td>
<td>0.43 ± 0.22</td>
</tr>
<tr>
<td></td>
<td>mGM</td>
<td>0.99 ± 0.11**</td>
<td>0.88 ± 0.18**</td>
<td>1.15 ± 0.14</td>
<td>0.31 ± 0.14</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.96 ± 0.13**</td>
<td>0.93 ± 0.15**</td>
<td>1.05 ± 0.10</td>
<td>0.33 ± 0.14</td>
</tr>
<tr>
<td>24 h</td>
<td>mWM</td>
<td>0.89 ± 0.10**</td>
<td>0.74 ± 0.13**</td>
<td>1.22 ± 0.18*</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>mGM</td>
<td>1.00 ± 1.00**</td>
<td>0.80 ± 0.12**</td>
<td>1.24 ± 0.11</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0.97 ± 0.97**</td>
<td>0.79 ± 0.11**</td>
<td>1.22 ± 0.12</td>
<td>–</td>
</tr>
<tr>
<td>8 days</td>
<td>mWM</td>
<td>1.05 ± 1.05**</td>
<td>1.23 ± 0.22</td>
<td>0.87 ± 0.09**</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>mGM</td>
<td>1.04 ± 0.90**</td>
<td>1.06 ± 0.12</td>
<td>1.00 ± 0.12*</td>
<td>–</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.05 ± 1.05**</td>
<td>1.12 ± 0.13</td>
<td>0.94 ± 0.11*</td>
<td>–</td>
</tr>
</tbody>
</table>

Average NAA/creatine, NAA/choline, choline/creatine, and lactate/creatine levels in all time-points pre-intoxication and post-intoxication are presented as mean value ± standard deviation.

mWM, voxels that represent mainly white matter; mGM, voxels that represent mainly gray matter; NAA, N-acetylaspartate.

*P < 0.05, **P < 0.005 (baseline vs. each of the time points values).

**FIG. 6.** Representative H&E staining of brain sections following paraoxon exposure (X’) compared with a nonintoxicated animal (X). Brain sections taken from the hippocampus (a,a’), hippocampal CA1 layer (b,b’), CA2 layer (c,c’), CA3 layer (d,d’), from the piriform cortex (E,F’), the thalamus (G,H’), and from the parietal cortex (I,J’) were processed for H&E staining as described in material and methods. Note the necrotic cells and vacuolation in b’, c’, f’, h’, and j’. In pictures a-a’, e-e’, g-g’, and i-i’, the magnification is X2 and the bar length is 1000 μm. In b-d, b’-d’, f-f’, h-h’, and j-j’, the magnification is X20 and the bar length is 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
hypothalamus (Spearman correlation coefficient of 0.88, \( P < 0.005 \)).

**DISCUSSION**

**T2 Alterations**

In our study, 3 h after the intoxication, parenchymal edema was detected as areas of elevated T2 values in the cortical and subcortical gray matter, mainly in the thalami. All these changes decreased gradually and returned to baseline values 8 days after the intoxication. A similar finding was reported by Gullapalli et al. with increased T2 values in the piriform cortex, hippocampus, thalamus, and amygdala of guinea pigs treated with 1 LD\textsubscript{50} of the warfare OP soman. Their imaging findings correlated with convulsive activity (19). Transient T2 increase in susceptible brain areas is also evident on other models of SE, such as pilocarpine and kainic acid-induced SE in rats and in a rat model of electrical stimulation of the amygdala (16,20–22). Moreover, a few studies used diffusion-weighted MRI, which is highly sensitive to cytotoxic edema, to assess brain damage after OP intoxication. In rodents treated with a convulsive dose of soman, low apparent diffusion coefficient values were described at 3 to 12 h after the exposure in the temporal cortex, in the amygdala, and in the thalamus. This decreased apparent diffusion coefficient values are suggestive of cytotoxic edema (11,13–15). Interestingly, Bhagat et al. described a decrease of T2 values in the hippocampus and in the thalamus in soman intoxicated rats which found 12 h postexposure and resolved after 24 h (13,14). Similar results were reported also by van Eijden et al. in the lithium-pilocarpine-induced SE in a rat model. Possible explanations for this decrease in T2 values were either a decline in extracellular water or an increased concentration of paramagnetic deoxyhemoglobin, which increases in situations in which the metabolic requirement for oxygen is not met by the delivery of oxygen such as in SE (discussed later) (23).

**Spectroscopic Changes**

To our knowledge, no previous study described in vivo MR spectroscopic changes of OP intoxicated animals. Our findings show significant decreased NAA/creatine and to a lesser degree NAA/choline levels as early as 3 h after the intoxication. Although NAA/creatine stayed low compared to baseline levels after 8 days, NAA/choline upraised to baseline levels. The progressive decrease of NAA/creatine and to lesser degree NAA/choline ratios in our paraoxon intoxicated rats are most likely attributed to decreased NAA due to neuronal death or dysfunction. Although previous neurochemical studies have described early increase in choline levels after OP intoxication (24,25), in our study, choline/creatine levels found to be decreased 3 h postintoxication (in mWM voxels). Following this early choline/creatine decrease, there was a statistically significant increase in mWM choline/creatine levels 24 h postintoxication which might represent transient high membrane metabolism and synthesis shortly after the cessation of the SE. Interestingly, on the 8th day choline/creatine ratio was significantly decreased compared to baseline level. This late change might represent a choline decrease, which was described in other SE studies (23), and is typical for a decrease in cell membrane turnover. A choline decrease might also explain the normalization of NAA/choline ratio on the 8th day due to both decreased NAA and choline. Ratios of peak integrals of metabolites to creatine are commonly used to analyze proton spectra in a clinical setting (26), van Eijden et al. showed no creatine peak changes in SE (23). However, some studies have shown a trend toward a decreased signal during the postictal period in both focal and generalized seizures (27). Creatine levels which may be decreased due to a depletion of tissue energy storage would only make the hypothesis of decreasing concentrations of choline and NAA stronger. Recently, Fauvelle et al. reported an ex vivo study of soman intoxicated mice, which were sacrificed in different time points, from 1 h to 7 days, and biopsies from the piriform cortex were analyzed for metabolic composition via proton high-resolution magnetic angle spinning nuclear MRS \((^1\text{H-HRMAS NMR})\). There they found a decrease in NAA levels in the piriform cortex, starting 4 h postintoxication and lasting for the 7 following days, similar to our results. The lowest value was obtained at 48 h. In concordance with our hypothesis, choline was increased during the first hours after the intoxication and eventually decreased by the 7th day after soman intoxication (28).

There have been two different hypotheses of the mechanism of OP-induced neuropathology. The first is the excitotoxic hypothesis which emphasizes the role of sustained seizure activity as the primary cause for the development of OP-induced neuropathology. A second hypothesis ascribes the neuropathology to hypoxia-anoxia-ischemia and emphasizes systemic factors (oxygenation, blood flow) as being primarily responsible for initiating the neuropathology (3). The systemic hypoxemia eventually results in cellular hypoxia in different body tissues of which brain and heart are most sensitive. Global ischemia and hypoxia of mature brain cause brain edema involved superficial and deep structures, primarily the cerebral cortex and basal ganglia. In Munkeby’s work in a piglet model, NAA/creatine and NAA/choline were reduced 7 h after deep hypoxic injury to the brain (29). In another animal model of global ischemia in rats in which ischemia was induced by bilateral carotid occlusion plus hypotension, NAA was decreased significantly in vulnerable regions, including in the cortex, striatum, hippocampus, and, to a lesser extent, in the thalamus. Areas with decreased NAA levels showed selective neuronal necrosis on histopathology studies and also showed a marked increase of lactate levels (30). The more severe involvement of the neuronal component stems from the concept of selective vulnerability, as neurons are known to be more vulnerable to oxygen deficiency than are oligodendroglia and astrocytes, whereas the microglia and the blood vessels are least vulnerable (31). In a case report of a human patient that suffered from diffuse hypoxic brain injury, reduction of cortical NAA/choline and NAA/creatine levels in MR spectroscopy was shown, suggesting a depletion of NAA. Measuring tissues hypoxemia in common medical practice is difficult. Lactate levels, which are not evident in normal
brain tissue, are thought to represent anaerobic metabolism. Interestingly, we found pathological MRS peak of lactate in all animals which was evident in the first MR session postintoxication (i.e., 3 h after the exposure). The lactate peak was in good correlation with overall mortality. In an ex vivo study of soman intoxicated rats, elevation of lactate levels was found only 24–72 h post-intoxication (28). In a piglet model of hypoxic-ischemic encephalopathy ratios of lactate/chocline and lactate/NAA were significantly higher after 7 h compared to baseline. The presence of necrosis in para-sagittal regions of the cerebral cortex and in the basal ganglia correlated well with the presence of lactate (29) To conclude, ischemic insult of which lactate elevation is the hallmark, is associated with brain parenchymal edema (increased T2 signal) and decreased NAA values (which probably represents neuronal dysfunction).

SE per se may cause metabolic and pathological changes in different brain tissues which may be apparent in imaging and pathological studies. In SE, there is a large increase in cerebral glucose metabolic rate and in oxygen consumption (7). Excitotoxic mechanisms mediated by glutamate receptors play a major role in this neuronal injury (7). Van Eijden et al. showed a decrease of NAA/creatine and NAA/choline, similar to our MRS findings, 2 h after lithium-pilocarpine-induced SE. At later time points, NAA/creatine continued to decrease, whereas NAA/choline and creatine/choline increased (23). In well-oxygenated animals with an adequate cardiovascular function, 2 h of SE causes moderate neuronal necrosis in the cerebral cortex, in the hippocampus, and in the thalamus (32). In rats, SE of 30 min duration or longer invariably caused infarction of the substantia nigra and of the globus pallidus (32). Brain NAA decrease was also reported by Najm et al. during post-ictal and inter-ictal phases in kainic-acid-induced seizures in rats. Interestingly, NAA/creatine levels were found to increase significantly during the ictal phase. The authors hypothesized that this rise might reflect increased cellular activity and metabolism resulting from kainic-acid excitotoxicity (33). In our study, similar to other SE models, lactate appeared nearly immediately after SE initiation (6,23,34). However, delayed and sustained elevated lactate levels were also reported in OP and non-OP-induced SE (28,33,35). Decreased NAA/creatine levels and elevated lactate levels were also reported in SE in humans (36,37).

MR Findings and Prognosis

Although the relatively homogenous clinical picture of the intoxicated animals, there was a significant variance in the severity of brain injury as shown both in the MRI and pathology studies. A similar finding was reported by Kadar et al. (9) after intoxicating rats with 1 LD50 of sarin, a potent military OP, i.e., only 70% of intoxicated animals showed brain lesions on histological studies. Brain lesions were seen only in 9 of 15 soman exposed monkeys after clinical convulsions and in only 1 of 21 that did not convulse (8).

No correlation was found between the 3 h MRI in which maximal parenchymal edema was shown, and brain histology studies. However, these finding might be biased as the least injured animals survived the experiment and their brain was sent to histology. Interestingly, there was a correlation between 8th day thalami T2 values and cortices damage. These findings might be consistent with retrograde neuronal degeneration because of thalamo-cortical fiber damage secondary to diffuse OP-induced cortical damage (38).

Animals who succumbed during the experiment showed extensive cortical edema, significant decrease in NAA/creatine and NAA/choline levels and elevation of lactate levels when compared with animals which survived during the experiment (mortality appeared 12 h and more after the intoxication). These MR findings were found as early as 3 h after the intoxication. Hence, these early MR findings might be used as early biomarkers for severity of intoxication and may help to predict the late prognosis of the intoxicated animals. Clinical use of MRI is promising, both for assessment of severity of brain injury and for overall prognosis of humans exposed to OPs.

Limitations

In our study, convulsive activity ceased after anesthesia was administered during first MR imaging. Volatile anesthetic agents including isoflurane have been well recognized for their potential neuroprotective properties since the 1960s (39). In humans, drug treatment of refractory generalized convulsive SE involves general anesthesia (40). Deep anesthesia probably slows the metabolic activity of the brain and thus stops seizures. Another limitation of this study includes a relatively small cohort of intoxicated animals.

CONCLUSIONS

This study is, to our knowledge, the first to study the brain of rats exposed to a convulsive dose of OP by means of MR spectroscopy. Our findings show that brain metabolic changes caused by paraoxon intoxication are obvious on MR scan already after 3 h. These changes are in correlation with short-term prognosis, i.e., survival. MR spectroscopy may, therefore, provide valuable noninvasive early biomarkers for the diagnosis, severity assessment of OP-induced neuropathology, and for the assessment of the efficacy of neuroprotective therapies.

In addition, our findings which have high similarity with imaging features of hypoxic-ischemic- and SE-induced brain damage might imply a common pathophysiological sequel in OP-induced neurotoxicity.

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