Practical Applications of MRI in Toxicologic Pathology

Part of the Imaging Workshop of the International Academy of Toxicologic Pathology (IATP)

Yael Schiffenbauer
Abraham Nyska
Invited Review

Toxicologic Pathology in the 21st Century

ROBERT A. HTTIN

Ettlin Consulting Ltd., Muenchenstein, Switzerland

ABSTRACT

Toxicology is and will be heavily influenced by advances in many scientific disciplines. For toxicologic pathology, particularly relevant are the increasing array of molecular methods providing deeper insights into toxicity pathways, in vivo imaging techniques visualizing toxicodynamics and more powerful computers anticipated to allow (partly) automated morphological diagnoses. It appears unlikely that, in a foreseeable future, animal studies can be replaced by in silico and in vitro studies or longer term in vivo studies by investigations of biomarkers including toxicogenomics of shorter term studies, though the importance of such approaches will continue to increase. In addition to changes based on scientific progress, the work of toxicopathologists is and will be affected by social and financial factors, among them stagnating budgets, globalization, and outsourcing. The number of toxicopathologists in North America, Europe, and the Far East is not expected to grow. Many toxicopathologists will likely spend less time at the microscope but will be more heavily involved in early research activities, imaging, and as generalists with a broad biological understanding in evaluation and management of toxicity. Toxicologic pathology will remain important and is indispensable for validation of new methods, quality assurance of established methods, and for areas without good alternative methods.
In Vivo Imaging- Don’t Choose, Fuse!
Advantages of MRI in Toxicology

• **Non invasive** - Permits longitudinal *in vivo* imaging to follow disease in the same animal (better statistics, less animals required)

• Can acquire numerous **digital slices** from whole fixed organs in any plane without destroying the specimen

• High **soft tissue contrast** as well as good bone visualization

• Provides a means to obtain **quantitative data**

  Provides complimentary information to conventional pathology- Better practice, safer drugs/products
MRI - A Psychological Barrier

- Expensive equipment
- Expensive maintenance
- Special facility required (shielded room)
- Safety issues (no metals around)
- Hard to operate
- For “MR gurus” only – High level of expertise required
Shift Happens – Compact MRI for Everyone

- Compact
- Quiet
- Affordable
- Easy to use
- Safe
- No special facility
- Maintenance-free
Complicated Software - No more
Tons of Parameters to Optimize... No more

Protocol Details of Fast Spin Echo 2D (Amos Fast Spin Echo)

**Advanced Parameters**

**Slice**
- Scan type (2D/3D): Fast3Dim
- Max # slices for this TR: 16
- Number of slices: 16
- Slice thickness (mm): 1
- Inter-slice gap (mm): 0.1
- Center slice position: 0
- Slice orientation: Coronal

**FOV/Resolution**
- Force eq. FOV horizon: Yes
- Hor. FOV (mm): 80
- Vert. FOV (mm): 80
- # phase encodings: 112
- # samples: 200
- FOV offset (vert, mm): 0
- FOV offset (hor, mm): 0

**Contrast (MRI)**
- Time to repeat (TR, ms): 4496
- Min TE: 0
- Time to echo (TE, ms): 77.854
- Apply inversion pulse: No
- Inversion time (TI, ms): 100.0
- Flip angle (deg): 90
- Apply diffusion sensitizing gradients: No
- Diffusion gradients: None

**Acquisition (k-space)**
- # excitations: 1
- Phase enc. direction: Horizontal
- Frequency direction: Vertical
- Dwell time (microsec): 25
- Partial Fourier: None
- External (respiration) trigger: None

**Reconstruction**
- Scaling method: ParSeries

**Perform calibrations**
- Frequency calibration: No
- Coil calibration: No
- Shim calibration: No
- RF calibration: No

**Display calibration data**
- Freq cal display mode: No
- Coil cal display mode: No
- Shim cal display mode: No
- RF cal display mode: No

**FSE parameters**
- Echo train length (ETL): 15
- Refocusing flip angle: 180
- FSE calibration: Retain
- FSE cal display mode: No
One Touch MRI - Ask. Touch. Answer.
Just Choose What You Need to Scan
Brain Protocols?
Heart protocols?
Source of Signal in MRI

- Tissue
- Cells
- Water/fat molecules
- Nuclear Spin
- Hydrogen atom

The diagram shows the relationship between tissue, cells, water/fat molecules, nuclear spin, and the hydrogen atom as sources of signal in MRI.
By changing the frequency, duration and timing of applied magnetic fields and radio frequency (rf) pulses, MRI can provide what are basically “MRI stains”

Most common “MRI stains” (Types of contrast) – T1 and T2
Any plane can be imaged
MRI Data Presentation – Slice through Animation
MRI Data Presentation – See through 3D Rendering (MIP)

Embryo 24 E17.5^24 E17.5
### Segmentation – Volume Calculation

<table>
<thead>
<tr>
<th>Sagittal</th>
<th>axial</th>
<th>coronal</th>
</tr>
</thead>
</table>

![Images of Sagittal, Axial, and Coronal views](image-url)
Segmentation – Volume Calculation

<table>
<thead>
<tr>
<th>ROI</th>
<th>Rat</th>
<th>Color</th>
<th>Voxels</th>
<th>Volume mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>rat embryo control E20</td>
<td>red</td>
<td>28910</td>
<td>97.5713</td>
</tr>
<tr>
<td>Heart</td>
<td>rat embryo control E20</td>
<td>blue</td>
<td>8218</td>
<td>27.7358</td>
</tr>
<tr>
<td>Liver</td>
<td>rat embryo control E20</td>
<td>cyan</td>
<td>84566</td>
<td>285.41</td>
</tr>
<tr>
<td>Brain</td>
<td>rat embryo control E20</td>
<td>magenta</td>
<td>38770</td>
<td>130.849</td>
</tr>
<tr>
<td>Left Kidney</td>
<td>rat embryo control E20</td>
<td>dark red</td>
<td>3044</td>
<td>10.2735</td>
</tr>
<tr>
<td>Right Kidney</td>
<td>rat embryo control E20</td>
<td>dark cyan</td>
<td>2745</td>
<td>9.26438</td>
</tr>
</tbody>
</table>
Scientific Collaboration

NIH - National Institute of Environmental Health Sciences

National Toxicology Program - U.S. Department of Health and Human Services

Harlan® - Harlan Biotech Israel Ltd.

The Hebrew University of Jerusalem

NeuroDerm

Eisai

RTC - Research Technology Centre·Iowa

Actelion
Methods

All scans performed on a M-series compact MRI by Aspect Imaging.

Animals:
- Anesthetized with isoflurane
- Heated
- Physiological monitoring

Fixed samples:
- In any fixative solution
- Fluorinerte
Models That Will be Presented

- Focal liver lesions
- Acute kidney injury
- Local Safety of SC formulations
- Biodegradable implant
- Brain tumor growth
- Rat lung fibrosis
- Neurotoxicity
Focal Hepatic Lesions

• **Model:** Mdr-/- mouse develops multiple focal hepatic lesions.

• **Objective:** Detect and measure volume of multiple focal lesions
Detection of Multiple Focal Lesions in Mouse Liver – *In Vivo* MRI

resolution 270 μm; slice thickness 1 mm; acquisition time 3.5 min
Multiple Focal Lesions in Mouse Liver

Ex vivo MRI

resolution 156 μm; slice thickness 0.7 mm; acquisition time 35 min
Segmentation of Lesions Based on Ex Vivo MRI
Quantification of Lesions Based on Ex Vivo MRI

- 15 distinctive lesions were detected
- The smallest lesion detected had a diameter of 0.6 mm
- The largest lesion had a diameter of 4.8 mm
- Total liver mass 2593 mm$^3$
- Total lesion mass 60.3 mm$^3$ (2.3%)
Segmentation of Lesions Based on Ex Vivo MRI

<table>
<thead>
<tr>
<th>ROI</th>
<th>Color</th>
<th>Voxels</th>
<th>Volume mm$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>red</td>
<td>701</td>
<td>2.6741</td>
</tr>
<tr>
<td>2</td>
<td>green</td>
<td>950</td>
<td>3.62396</td>
</tr>
<tr>
<td>3</td>
<td>blue</td>
<td>1123</td>
<td>4.28391</td>
</tr>
<tr>
<td>4</td>
<td>cyan</td>
<td>342</td>
<td>1.30463</td>
</tr>
<tr>
<td>5</td>
<td>magenta</td>
<td>2047</td>
<td>7.80869</td>
</tr>
<tr>
<td>6</td>
<td>yellow</td>
<td>831</td>
<td>3.17001</td>
</tr>
<tr>
<td>7</td>
<td>dark red</td>
<td>2271</td>
<td>8.66318</td>
</tr>
<tr>
<td>8</td>
<td>dark green</td>
<td>1961</td>
<td>7.48062</td>
</tr>
<tr>
<td>9</td>
<td>dark blue</td>
<td>204</td>
<td>0.778198</td>
</tr>
<tr>
<td>10</td>
<td>dark cyan</td>
<td>245</td>
<td>0.934601</td>
</tr>
<tr>
<td>11</td>
<td>tomato</td>
<td>201</td>
<td>0.766754</td>
</tr>
<tr>
<td>12</td>
<td>maroon</td>
<td>197</td>
<td>0.751495</td>
</tr>
<tr>
<td>13</td>
<td>orchid</td>
<td>99</td>
<td>0.377655</td>
</tr>
<tr>
<td>14</td>
<td>peach puff</td>
<td>143</td>
<td>0.545502</td>
</tr>
<tr>
<td>15</td>
<td>light sea green</td>
<td>152</td>
<td>0.579834</td>
</tr>
</tbody>
</table>
Classification of Liver Lesions as Focal Fatty Changes by Histopathology
Summary & Comment

- In vivo and ex vivo MRI evaluation were effective in identifying the location and measuring the volume of focal changes in the liver.

- This approach using in vivo MRI would allow for following lesion development over time.

- In this study the MRI was done after lesions were fully developed, however, longitudinal studies using in vivo MRI would easily be feasible in this model.
Rhabdomyolysis-Induced Acute Kidney Injury (AKI) in Mouse

Mice Model of Glycerol-Induced AKI

CB6F1 Mice
IM 50% Glycerol

MRI + Blood samples
15 8 3 0 Days

Histopathology – Day 3

cortex
medulla
control
affected

aspect imaging
Control vs Affected Kidney

In Vivo MRI

Day 3

- Loss of contrast
- Enlarged kidneys

Control vs Affected Kidney

In Vivo MRI

Day 3

- Loss of contrast
- Enlarged kidneys

resolution 234 μm; slice thickness 1mm; acquisition time 10 min

cortex

medulla

aspectimaging
Following Disease Progression

*In Vivo MRI*

Contrast lost and kidney enlargement

Day 3

Day 0

Day 8

Day 15

Contrast and size recovered
Control vs Affected Kidney

Ex Vivo MRI

Resolution 117 μm; slice thickness 0.5 mm; acquisition time 56 min

Control

Affected

Loss of contrast

cortex
medulla
papila
MRI & Histology – Control Kidney

Ex Vivo

cortex X 200

medulla X 200
MRI & Histology – Affected Kidney

Ex Vivo

cortex X 200

medulla X 200
Summary of Findings & Comment

- *In-vivo* and *ex-vivo* MRI were effective in identifying alterations in the cortex and medulla. History: Maximal extent of cortical necrosis and medullary hyaline cast formation.

- *In-vivo* and *ex-vivo* MRI confirmed organ recovery. History: The previously necrotic tubules were replaced by regeneration.
Local Safety of Subcutaneous Formulations

• **Model:** In this study, subcutaneous lesions were analyzed by MRI 2 weeks after a 24-hour continuous infusion of different formulations.

• **Objective of the experiment:** This was a feasibility study for application of the *Ex-Vivo MRI* in order to evaluate the subcutaneous toxic effects induced at the injection site of test compounds.
Subcutaneous Drug Injection Into Pig Skin
MRI vs. Histology

MRI (T1)  vs.  Histology
H&E Histopathology

Blue = Multifocal areas of fat necrosis & associated inflammation
Red = Normal adipose tissue
Subcutaneous Drug Injection Into Pig Skin - *Ex Vivo* MRI
Segmentation and Quantification of Affected Volume - Ex Vivo MRI

Affected Volume 2200 mm³
Summary & Comment

• **Ex vivo MRI** was effective in identifying the location and quantifying the extent of subcutaneous necrosis and inflammation caused by different formulations.

• Applying this method on fixed tissues samples derived from different dose formulations provides a quantitative determination of relative irritancy of different injected formulations.
Biodegradable Implanted Device

- **Model:** A double layer of a 5x5 mm² device was implanted in the right paralumbar muscle of Sprague Dawley rats.

- A plastic bead was implanted subcutaneously just over the device to enable accurate localization and follow-up of the implantation site.

- **Objective:** Evaluation of *in vivo* MRI as a tool for assessment of degradation of a bio-degradable device.
**In Vivo MRI of Implanted Device**

<table>
<thead>
<tr>
<th></th>
<th>Day 5</th>
<th>Day 30</th>
<th>Day 60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T1</strong></td>
<td><img src="image" alt="Day 5 T1" /></td>
<td><img src="image" alt="Day 30 T1" /></td>
<td><img src="image" alt="Day 60 T1" /></td>
</tr>
<tr>
<td></td>
<td>bead</td>
<td>implant</td>
<td></td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td><img src="image" alt="Day 5 T2" /></td>
<td><img src="image" alt="Day 30 T2" /></td>
<td><img src="image" alt="Day 60 T2" /></td>
</tr>
<tr>
<td></td>
<td>Inflammation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**aspect imaging**
Ex Vivo MRI of Implanted Device
Segmentation and Quantification

Volume of device: **32.2 mm³** at day 60
Histopathology of Implantation Site After 60 Days

- **mature connective tissue capsule**
- **cavity of device**
Longitudinal Growth of a Brain Tumor

- **Model:** GI-261 glioma cells stereotactically injected into the right brain hemisphere of CB6F1 mice

- **Objective:** Longitudinal evaluation of tumor growth
Longitudinal Evaluation of Tumor Growth

*In Vivo MRI*

<table>
<thead>
<tr>
<th>Day 15</th>
<th>Day 17</th>
<th>Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>axial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>coronal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exponential tumor growth

resolution 156 μm; slice thickness 1 mm; acquisition time 13 min
Tumor Segmentation – *Ex Vivo* MRI

Day 17 – coronal

Tumor volume 6.6 mm$^3$

Injection site

Injection site
<table>
<thead>
<tr>
<th>Day</th>
<th>Ex Vivo MRI</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td><img src="image1" alt="MRI Day 15" /></td>
<td><img src="image2" alt="Histology Day 15" /></td>
</tr>
<tr>
<td>20</td>
<td><img src="image3" alt="MRI Day 20" /></td>
<td><img src="image4" alt="Histology Day 20" /></td>
</tr>
</tbody>
</table>
Summary & Comment

• *In-vivo* and *ex-vivo* MRI evaluation provided a way to follow the time-related growth of an induced tumor in the brain and to determine the volume of the tumor.

• This model demonstrates the utility of using MRI for longitudinal studies and would be useful for testing the efficacy of anti-cancer drugs.
Rat Lung Fibrosis

- **Model:** Single intratracheal instillation of bleomycin into 6 week-old Sprague Dawley rats

- **Objective:** Monitor lung fibrosis in rats using *in vivo* and *ex vivo* MRI as a tool for following temporal progression of the pathological process
Rat Lung Control vs. Fibrosis

*In Vivo MRI*

**Day 11 Post Instillation**

control

fibrotic

resolution 274 μm; slice thickness 1.2 mm; acquisition time 4.5 min
Time Course of Disease

*In Vivo MRI*

<table>
<thead>
<tr>
<th>Day</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>204 gr</td>
</tr>
<tr>
<td>Day 8</td>
<td>183 gr</td>
</tr>
<tr>
<td>Day 11</td>
<td>190 gr</td>
</tr>
<tr>
<td>Day 14</td>
<td>228 gr</td>
</tr>
</tbody>
</table>
Control rat lungs inflated with air vs instilled in formalin (the ones instilled with formalin are a bit brighter)
<table>
<thead>
<tr>
<th>BLM air</th>
<th>BLM formalin</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

BLM rat lungs inflated with air vs instilled in formalin (fibrosis in “air” lungs is more visible on the darker background)
Control vs. Fibrosis – *Ex Vivo* MRI

Day 11 Post Instillation

Control

Fibrosis
Fibrotic Rat Lung – Volume Quantification Based on *Ex Vivo* MRI

Day 11 Post Instillation

**3D rendering**

**3D rendering + segmentation**

Connective Tissue: **1267 mm³**
Normal Tissue: **1396 mm³**

aspectimaging
Histology – Masson’s Trichrome

<table>
<thead>
<tr>
<th></th>
<th>Low magnification</th>
<th>high magnification</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td><img src="image1" alt="Control low magnification" /></td>
<td><img src="image2" alt="Control high magnification" /></td>
</tr>
<tr>
<td>fibrosis</td>
<td><img src="image3" alt="Fibrosis low magnification" /></td>
<td><img src="image4" alt="Fibrosis high magnification" /></td>
</tr>
</tbody>
</table>
Summary & Comment

• *In vivo* MRI provides a longitudinal evaluation of pulmonary disease progression and regression

• *Ex vivo* MRI in combination with histology provides a quantitative assessment of the components of the interstitial thickening

• Based on the ability to quantify the extent of disease, different therapeutic modalities can be compared for their effectiveness
Pilocarpine- Induced Status Epilepticus

• **Model**: SD male rats treated with LiCl followed by Pilocarpine, a muscarinic cholinergic agonist and accepted model to induce status epilepticus and morphologic damage in rat brain.

• **Expected outcome**: Neuronal cell degeneration /necrosis

![Brain Image]

- **Dark areas**: Severe
- **Hatched areas**: Moderate
- **Dotted areas**: Slight
Control vs Pilocarpine
Ex vivo MRI (T1)

1: Piriform cortex
2: Lateral thalamic nucleus
3: Posterior hypothalamic nucleus
4: Hippocampus
5: Caudate putamen
Control vs Pilocarpine – H&E

1: Piriform cortex
2: Lateral thalamic nucleus
3: Posterior hypothalamic nucleus
4: Hippocampus
5: Caudate putamen
Control vs Pilocarpine
Ex vivo MRI (T2)

control

Pilocarpine
Bars = 20 μm

Pathologic Grade; 1+: Slight, 2+: Moderate, 3+: Marked

1. Piriform cortex
2. Lateral thalamic nucleus

Control

Pilocarpine
Control

Pilocarpine

3. Posterior hypothalamic nucleus

4. Hippocampus

Control

2+
Summary

- MRI imaging demonstrated areas of high T1 and low T2 signals compared to controls in the piriform cortex, lateral thalamic nucleus, posterior paraventricular thalamic nucleus, and posterior hypothalamic nucleus of the cerebrum.

- Histopathology showed neuronal cell degeneration and necrosis accompanied by gliosis in these areas.

- MRI analysis of fixed organs before routine slide preparation could provide useful information for histopathologic evaluation in preclinical toxicity studies.
Neurotoxicity induced with Kainic acid

Base line

3rd day
T2 maps following treatment with Kainic acid

Baseline:

3<sup>rd</sup> day
T2 maps following Kainic acid: 1T comparable with 7T

**1 Tesla**
- Control
- KA (Edema in insular cortex)

**7 Tesla**
- Control
- Edema
- Neurodegeneration in hippocampus
- Edema in insular cortex

Note the disarrayed cellular layer in the CA3 region of the hippocampus, suggestive of neurodegeneration even in the absence of silver deposition.
Neuronal degeneration, necrosis and vacuolation in hippocampus
Intramyelinic edema, neuronal necrosis and gliosis in amygdaloid nuclei region
Conclusions: “collect Smart sections...”. “..The application of full brain MRI imaging that informs neuropathology offers the potential to dramatically improve detection of neurotoxicity produced by new drugs and facilitate new drug development, review and approval processes, and to qualify an imaging biomarker of neuropathology.”
MRI-based Histology- **Smart Sections**
Added Value for Lesion Evaluation

- Localize the lesions
- Count the lesions
- Measure lesions volume
- Longitudinal in-vivo follow-up in the same animal
- Information about homogeneity of the lesions