The value of BOLD MR imaging in a rat model of chronic pancreatitis: a preliminary study

Poster No.: C-0976
Congress: ECR 2014
Type: Scientific Exhibit
Authors: R. Chai, K. Ren, Z. Chu; Shenyang/CN
Keywords: Abdomen, MR, Treatment effects
DOI: 10.1594/ecr2014/C-0976

Any information contained in this pdf file is automatically generated from digital material submitted to EPOS by third parties in the form of scientific presentations. References to any names, marks, products, or services of third parties or hypertext links to third-party sites or information are provided solely as a convenience to you and do not in any way constitute or imply ECR's endorsement, sponsorship or recommendation of the third party, information, product or service. ECR is not responsible for the content of these pages and does not make any representations regarding the content or accuracy of material in this file.

As per copyright regulations, any unauthorised use of the material or parts thereof as well as commercial reproduction or multiple distribution by any traditional or electronically based reproduction/publication method is strictly prohibited.

You agree to defend, indemnify, and hold ECR harmless from and against any and all claims, damages, costs, and expenses, including attorneys' fees, arising from or related to your use of these pages.

Please note: Links to movies, ppt slideshows and any other multimedia files are not available in the pdf version of presentations.

www.myESR.org
Aims and objectives

Chronic pancreatitis (CP) is a disease of the pancreas in which recurrent episodes of inflammation lead to replacement of the pancreatic parenchyma with fibrotic tissue.[1] CP gives rise to treatable complications including endocrine and exocrine pancreatic insufficiency, acute episodes of inflammation, pseudocyst formation, cholestasis, and an increased risk of pancreatic carcinoma.[2] As in other diseases, the histological examination is considered the gold standard of CP diagnosis, however, the pancreatic biopsy is rarely possible and the heterogeneity of the lesion obscures the accurate diagnosis.

Four imaging procedures are commonly used for the evaluation of CP: ultrasound, CT, endoscopic retrograde cholangio-pancreatography (ERCP), and MRI. However, their diagnostic performances are often compromised by various shortcomings. The performance of ultrasound is operator dependent and easy to be affected by peripheral tissues. CT necessitates exposing the patient to ionizing radiation and iodinated contrast agents. In addition, CT plays a limited role in mild CP with little morphology and density changes. ERCP is invasive, requires patient sedation, and can itself result in acute pancreatitis.[3] MR, because of high-resolution to soft tissue and no ionizing radiation, is valuable for assessing the full spectrum of pancreatic diseases and is growing in popularity.[4] However, gadolinium-based contrast agents are associated with allergic reactions and nephrogenic systemic fibrosis in certain patient groups. Moreover, it is accepted that the mild CP is minimal in histological changes and normal in imaging. Thus, it is poor in detecting CP at an early stage through common imaging procedures. The development of a safe, noninvasive, and reproducible imaging method to diagnose CP at early stage would be necessary. The present work was undertaken to evaluate the potential for a new MRI-based technique that may be capable of providing functional information.

One potential method useful for in vivo functional assessment of tissue is blood oxygen level-dependent (BOLD) MR imaging. BOLD MR imaging has been applied to monitor the effects of changes in blood oxygenation, works through modulating the T2*-weighted signal by changing the ratio of paramagnetic deoxyhemoglobin (deoxyHb) to diamagnetic oxyhemoglobin (oxyHb) in the blood.[5, 6,7] DeoxyHb creates magnetic susceptibility perturbations around blood vessels, increasing the MR relaxation time R2*. Gradient-recalled echo (GRE) MR sequences are sensitive to the tissue deoxyHb concentration through R2*. The initiation and progression of CP involve a series of physiological factors, such as blood flow, blood volume, and tissue metabolism,[8] which may induce a change in the ratio of deoxyHb to oxyHb. Hence, pancreatic R2* may provide an index related to tissue oxygenation of CP.
Although BOLD MRI was first observed in rat brain studies[5], it has been used to assess tissue oxygenation in other bodily organs, such as the kidney[9], heart[10], skeletal muscle[11], and liver[7, 12]. However, there has been no report about application of BOLD MRI on pancreas. The principal aim of our study was to investigate the feasibility of BOLD MRI as a noninvasive, nonenhanced imaging method to evaluate the pancreatic functional information in a rat model of dibutyltin dichloride (DBTC)-induced CP.

**Methods and materials**

**Animal Model**

All experiments were performed in accordance with the guidance suggestions for the care and use of laboratory animals published by the Ministry of Science and Technology of the People's Republic of China in 2006[13]. Thirty five adult male Wistar rats that initially weighed 170-200g were used for the experiments. For intravenous administration, 400mg DBTC was first dissolved in 400ml ethanol and then mixed with 100ml saline. The DBTC solution was injected through tail vein at a dose of 0.8mg/kg body weight in thirty rats. Five rats that received equivalent solvent served as control animals.

**MR Imaging and Postprocessing**

MR imaging was performed in five control rats at day 28, in four rats at five days and seven days each after DBTC administration, and in eight rats at 14 days and 28 days each after DBTC administration. All MR imaging examinations were performed by using a 3.0-T clinical MR unit with a wrist-joint coil. Anesthesia was induced with an intramuscular injection of 3% pentobarbital sodium at a dose of 0.4ml/kg, and then supine fixed to a self-made plastic frame. MR images of the entire abdomen were acquired at coronal T2-weighted, transverse T1-weighted and T2-weighted. To quantify R2*, transverse multigradient-echo images were acquired by using the following parameters: repetition time msec/echo time msec, 140/16; echo train length, 2; flip angle, 30°; number of sections, six; section thickness, 1 mm; average number of signals acquired, eight; number of phase-encoding steps, 256; field of view, 18×18 cm; and temporal resolution, 151 sec while the rats free breathing.

Image postprocessing was performed on workstation by using R2* Map software. Before being processed, all of the images were visually inspected to ensure that none of them was corrupted with respiratory motion-induced artifacts. On the R2* images obtained in each animal, a region of interest placed in the pancreatic parenchyma on each section while excluding blood vessels was drawn. For each animal, the value of R2* was measured.
Histologic Evaluation

After the MR examinations, the histologic slices excised from the pancreatic parenchyma of each rat were fixed in 10% buffered formaldehyde solution and embedded in paraffin. Haematoxylin and eosin staining was used to evaluate the histological features and fibrosis. The extent and distribution of fibrosis of the pancreas were graded according to a score system depicted in Table 1. Mild and focal perilobular fibrosis was considered the lowest degree of pancreatic fibrosis (score 1) and diffuse perilobular and intralobular fibrosis the highest degree (score 12). Perilobular fibrosis was defined as the presence of connective tissue within the interlobular spaces. Intralobular fibrosis was defined as an extension of the perilobular fibrosis into the acinar lobules with partial (mild: 10-40%; moderate: 40-80%) or (almost) complete (severe: 80-100%) fibrous replacement of the acinar cells.[14, 15, 16] The severity of CP was determined on the histopathology as three classes, which were: (1) sparse inflammatory infiltrate, with slight degeneration of acinar cells and little intralobular fibrosis (mild CP); (2) inflammatory infiltrates within the lobules, acinar cell atrophy, and moderate intralobular fibrosis (moderate CP); (3) almost complete replacement of pancreatic exocrine tissue by fibrosis (severe CP).[14, 17]

Statistical Analysis

The rats were divided into 6 groups according to the time of administration. The mean and SD for the R2* and degree of fibrosis were calculated. R2* of every group was analyzed with t-test. Statistical analysis results were considered to be significant at P<0.05. All statistical analyses were performed by using SPSS 13.0 software.

Results

Animal models and histopathology

Six experimental rats died of complications after DBTC administration. In the remaining 29 rats, the control and experimental animals were autopsied after MR imaging. All the control rats had normal pancreas on gross findings and histology (Fig. 1). One rat at 7 days and five rats at 14 days after DBTC administration were found pancreatic fibrosis of score 1, two rats at 14 days and four rats at 28 days of score 2, two rats of score 3 and two rats of score 4 at 28 days each. The distribution of fibrosis was heterogeneous in the pancreas. In addition, the intralobular fibrosis appeared posterior to the perilobular fibrosis and was observed in only two rats at 28 days after DBTC administration. The inflammatory cell infiltration and acinar cell atrophy were most severe at the 7-day group, and decreased at the 14-day group and the 28-day group. Except the two rats with
pancreatic fibrosis of score 4 were graded as moderate CP (Fig. 2), all the other with pancreatic fibrosis deposition were graded as mild CP.

**MRI findings**

In the routine MR imaging, we found a dilation of pancreatic duct in only one 28-day rat, and others with histological fibrosis all had little morphological and signal changes. However, on the postprocessed R2* maps, we observed an obvious color change from blue to red with the degree of CP. (Fig. 3)

The measured R2* of every group increased with the time after DBTC exposure (Table 2). There were significant differences in R2* between the rats of control group and 5-day group (P = .004), between the rats of 5-day group and 7-day group (P = .015), between the 5-day group and the 14-day group (P#.001), and between the 14-day group and the 28-day group (P#.001). However, no significant difference was found between the rats of 7-day group and 14-day group (P = .131).

**Images for this section:**
### Table 1: Scoring System for the Evaluation of Fibrosis in CP

<table>
<thead>
<tr>
<th>Pattern of fibrosis</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perilobular fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Intralobular fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Diffuse</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
Fig. 1. Normal pancreas of a control rat. (A) The pancreas was pink and soft (as arrow). (B) There was no fibrosis deposition nor inflammatory cell infiltration on haematoxylin and eosin stained slice (× 100).

Fig. 1: Normal pancreas of a control rat
Fig. 2: Moderate pancreatitis at 28 days after DBTC administration. (A) The pancreas was pale and tough (as arrow). (B) There were abundant inflammatory cells and fibrosis on haematoxylin and eosin stained slice (×400).
Fig. 3: Figure 3. R2* Images  The rainbow bar on the left reflects the R2* value. The tissue in red color has a higher R2* value than in blue color, and thus low oxygenation level. (A) The normal pancreas appeared as blue in the R2* image. The color of pancreas gradually changed from green to orange red at B,C and D, which was corresponding to the rats from the 7-day group, the 14-day group and the 28-day group.
<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>R2*(sec⁻¹)¹</th>
<th>Fibrosis Score¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>6.56±3.65</td>
<td>0</td>
</tr>
<tr>
<td>5-day group</td>
<td>4</td>
<td>10.44±1.57</td>
<td>0</td>
</tr>
<tr>
<td>7-day group</td>
<td>4</td>
<td>13.44±3.37</td>
<td>0.25±0.5</td>
</tr>
<tr>
<td>14-day group</td>
<td>8</td>
<td>14.88±3.42</td>
<td>1.13±0.64</td>
</tr>
<tr>
<td>28-day group</td>
<td>8</td>
<td>21.62±4.88</td>
<td>2.75±0.89</td>
</tr>
</tbody>
</table>

¹ Data are means±standard deviations.

Table 2: R2* Values and Fibrosis Scores of Different Groups
Conclusion

DBTC has been reported to induce a chronic course of inflammation accompanied by the development of pancreatic fibrosis in rats in dependence on the time and dose after single administration.[18, 19] Thus, this model seems to be suited to evaluate the changes of blood oxygen level during initiation and progression of CP.

In our study, the degree of CP and the fibrosis score both increased with time after DBTC administration. But in the routine MR imaging, we found a dilation of pancreatic duct in only one 28-day rat, while others with histological fibrosis all had little morphological and signal changes. It revealed that routine MR imaging plays a limited role in diagnosis of mild CP. However, the R2* map could directly reflect the hypoxia condition of the pancreatic tissue and help detect the disease.

There were significant differences in R2* between the rats of control group and 5-day group, between the rats of 5-day group and 7-day group, between the 5-day group and the 14-day group, and between the 14-day group and the 28-day group. These findings suggest a reduced blood oxygen level in the development of disease. This may result from (a) fibrosis in the pancreas which may lead to the distortion, compression, and even obliteration of the vasculature; (b) decreased functional pancreatic flow due to the increased resistance of pancreatic parenchyma; or (c) capillary rarefication due to the acinar atrophy, inflammatory response and fibrosis.[8] However, the R2* between the 7-day group and the 14-day group were insignificant. There was little fibrosis but relatively severe inflammatory response at 7 days after DBTC. This may lead to an increase of blood flow and oxygen consumption, thus result in an increase of R2*. Given the promising results of our preliminary study and the noninvasiveness of the BOLD MRI method, translational studies involving patients with CP are potential.

There were several limitations in our study. First, histopathological changes, blood flow and the macroscopic magnetic field inhomogeneities all influence the R2* as an integral. [20] We did not evaluate their contribution respectively. Future studies are needed to investigate the relationship between R2* and the above factors. Second, we did not analyze the relationship between R2* and fibrosis degree. Because of the small sample, we only compared the R2* among different groups according to time after DBTC. In future studies, the correlation between R2* and histological parameters should be analyzed. Finally, we measured the R2* for the entire pancreas rather than for specific pancreatic regions on each image section. Because the shape of rat pancreas is patchy and it was difficult to match the MR images with the histologic specimens. However, heterogeneity of fibrosis distribution was observed and this may lead to differences in BOLD response in specific regions. Future studies to further investigate these intrapancreatic differences should be performed.
For the first time, we provide an evidence of the feasibility of BOLD MR in diagnosis of CP. The 3.0-T MR unit and multigradient-echo sequences are readily available on many medical institutions. In addition, the $R_2^*$ value is quantitatively obtainable by using $R_2^*$ map software. Thus, it is feasible to evaluate the changes of the oxygenation level in rat models by BOLD MR.

In conclusion, BOLD MRI is a promising and noninvasive method for the early diagnosis of experimental CP. $R_2^*$ had a significant increase during the development of the disease and could reflect the decreased oxygenation level. Future studies to evaluate the effectiveness of BOLD MR imaging in patients with CP are potential.

**Personal information**

R. Chai, M.D.

Department of Radiology, the First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China;

chairuimei@sina.cn

K. Ren, M. D., Ph. D.

Department of Radiology, the First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China;

renke815@sina.com

Z. Chu, M. A.

Siemens Healthacre Customer Service Sector, Siemens, Shenyang, China.

**References**


13 The Ministry of Science and Technology of the People's Republic of China. Guidance suggestions for the care and use of laboratory animals. 2006-09-30


