Original contribution

Assessing the correlation between the degree of disc degeneration on the Pfirrmann scale and the metabolites identified in HR-MAS NMR spectroscopy

Maciej Radek a,⁎, Barbara Pacholczyk-Sienicka b,⁎, Stefan Jankowski b,1, Łukasz Albrecht b, Magdalena Grodzka c, Adam Depta d,e, Andrzej Radek a

⁎ Department of Neurosurgery and Peripheral Nerve Surgery, WAM University Hospital, Central Veterans' Hospital of the Medical University of Łódź, Poland
b Institute of Organic Chemistry, Faculty of Chemistry, University of Technology, Łódź, Poland
c Department of Radiological and Isotopic Diagnostics and Therapy, Medical University of Łódź, Poland
d Department of Management, Technical University of Łódź, Poland
e Department of Health Care Financing, Medical University of Łódź, Poland

A R T I C L E  I N F O

Article history:
Received 6 September 2015
Revised 10 December 2015
Accepted 12 December 2015

Keywords:
Degenerative disc disease
Metabolic profile
MRI spectroscopy
HR MAS NMR

A B S T R A C T

Objective: The objective of this study is to assess the correlation between the degree of degeneration of lumbar discs according to the Pfirrmann classification system and the concentrations of metabolites determined by means of 1 H high-resolution magic angle spinning nuclear magnetic resonance (1 H HR MAS NMR) spectroscopy.

Materials and methods: Twenty-six human intervertebral lumbar discs that were operated on due to degenerative disease were analyzed. Routine preoperative 1.5 T, T2-weighed magnetic resonance (MR) images were used to classify the cases according to the Pfirrmann classification system. In all the cases, during microdiscectomy, the fragments of the annulus fibrosus and nucleus pulposus were harvested and their metabolic profile was examined by means of 1 H HR MAS. The grades of disc degeneration on the Pfirrmann scale were correlated with the metabolite concentrations.

Results: Spectral analyses of the intervertebral discs with Pfirrmann grades IV and V demonstrated significantly higher concentrations of creatine, glycine, hydroxyproline, alanine, leucine, valine, acetate, isoleucine, α,β-glucose, and myo-inositol, and a lower intensity of the N-acetyl peak of chondroitin sulfate, compared to the spectra with Pfirrmann grade III.

Conclusion: Our results demonstrate correlations between metabolite concentrations and the degree of lumbar disc degeneration assessed using the Pfirrmann grading system and provide another step toward the potential use of in vivo MR spectroscopy for investigation of biomarkers in lumbar disc degeneration.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Lumbar degenerative disc disease is a common and serious problem most often affecting people after the third decade of life, thus being a very important issue for society due to the high overall costs, including work incapacity and treatment costs. An upright body position, increase in body weight and a change toward a sedentary lifestyle causes more stress to the discs and accelerates the biochemical and biomechanical changes, in both the annulus fibrosus and the nucleus pulposus. Disc degeneration seems to be a process of premature aging. The age-related changes of the disc include dehydration, fibrosis of the nucleus pulposus, disorganization of the annulus fibrosus lamellae, and thinning and calcification of endplates [1,2]. Dehydration of intervertebral discs increases with ageing and causes changes in its structure that may constrain the transport of nutrients. The decreased levels of nutrients are believed to be one of the main causes of disc degradation. Intervertebral discs are the largest avascular tissues in the body and are supplied with small molecules such as oxygen and glucose from the endplates by diffusion or convection [3,4,5]. A lack of sufficient cellular nutrition may lead to cell death and increased matrix degradation, resulting in disc degeneration [5].

Nowadays the best method of disc investigation is magnetic resonance imaging (MRI). There are many systems of classification for the changes that can be seen on MR images and their relation to the clinical symptoms. In our practice we use the Pfirrmann grading system [6], which quite uniquely focuses on the structure of the disc. The level of degeneration is assessed on T2-weighted images based on the changes in the MRI signal intensity, disc structure, the distinction between the nucleus and the annulus, and disc height. There are five
grades, I–V: Grade I represents a healthy looking disc, with homogeneous structure, a bright hyperintense nucleus signal, and normal disc height. Grade II: the disc is inhomogeneous with a hyperintense signal and a good distinction between the annulus and the nucleus. The disc height is normal. Hypointense bands are visible. Grade III: the structure of the disc is inhomogeneous, the signal intensity is intermediate, the distinction between the annulus and the nucleus is unclear, and the disc height is normal or decreased. Grade IV: the disc is inhomogeneous, with hypointense signal intensity. It is impossible to distinguish between the annulus and the nucleus. The disc height is normal or decreased moderately. Grade V: the disc is inhomogeneous, with hypointense signal intensity. It is impossible to distinguish the nucleus from the annulus. The disc is collapsed [6] (Fig. 1). This grading system focuses on the disc structure and is well used in clinical practice, but there is a lack of information about biochemical changes in the disc.

The purpose of this study was to evaluate the correlation between grades of degeneration of the discs according to the Pfirrmann classification system and the chemical composition of the discs determined by means of 1H HR MAS NMR spectroscopy. We believe that in the future high-resolution MRI scanners will allow for spectroscopy of intervertebral discs. Identifying the levels of metabolites in different degrees of degeneration of the discs and combining the results with outcome of the patients can help establish better treatment methods; surgical vs. medical and rehabilitation or implementation of regenerative medicine.

2. Materials and methods

2.1. Preoperative MRI assessment

In all cases, the preoperative 1.5 T MRI, T2-weighted images were assessed by an independent, experienced radiologist and classified according to the Pfirrmann grading system. In our group, we only had patients with grades III (n = 4), IV (n = 16) and V (n = 6).

2.2. Disc tissue samples

All tissue samples were harvested from patients who underwent intervertebral disc surgery at the WAM University Hospital, Central Veterans' Hospital of the Medical University of Lodz, Poland. This project was approved by the local ethics committee (approval no. RNN/355/12/KB). There were 26 disc specimens harvested from 26 patients. The mean age of the patients was 46.5 ± 13.7 (SD) years (range 18–72 years). In all cases, the surgical technique used was microdiscectomy. After exposing the posterior aspect of the disc...
under the microscope, the annulus was fenestrated and a 3 × 3 mm fragment was taken with a punch. The sample was put into a plastic container with the patient’s data, and the level of the disc noted on it. In the next step, the tissue from the deeper layer was harvested and put into a separate container. All tissue samples after the surgery were immediately frozen and then stored at −80 °C until HR MAS analysis. Before the HR MAS analysis, all samples were cut to fit the 4 mm zirconium HR MAS rotor (total sample volume 50 μL).

2.3. HR MAS experiments

The discs samples were analyzed by means of 1H HR MAS technique. The HR MAS spectra were recorded on a Bruker Avance II Plus 700 MHz spectrometer, which was equipped with a 4 mm 1H/13C HR MAS probe. Phosphate-buffered saline (PBS 0.1 M, pH 7.4, 30 l L) made with deuterium oxide and containing 3.8 mM TSP (sodium-30-trimethylsilylpropionate-2,2,3,3- d4) was added to each sample. The TSP peak at 0 ppm was used as a chemical shift standard and a linear baseline correction was applied. Detailed information about the measurements is provided in our previous paper[7].

3. Results

Representative ex vivo 1H HR MAS NMR spectra of the degenerated disc tissues with Pfirrmann grades III, IV and V are shown in Fig. 2.

The spectra of intervertebral discs with Pfirrmann grades IV and V were characterized by higher concentrations of lactate, creatine, glycine, hydroxyproline, alanine, leucine, valine, acetate, isoleucine, α,β-glucose, myo-inositol, and 2-propanol, and a lower concentration of the N-acetyl peak of chondroitin sulfate, compared to spectra with Pfirrmann grade III. This observation was associated with a degenerative process, when collagen and proteoglycan macromolecules degrade into their mobile chemical constituents such as amino acids and sugars. The changes in concentrations of these metabolites along with an increasing level of degeneration on the Pfirrmann scale are shown in Fig. 3.

There was also an observation of a strong correlation between patients’ age and increasing level of disc degeneration (correlation coefficient r = 0.51, p = 0.01), which confirmed that the degeneration process was associated with ageing.

Moreover, all possible metabolite ratios were analyzed for the three groups of patients with different Pfirrmann grades. The Gly/Cr and Val/Leu ratios showed statistically significant differences among all three Pfirrmann grades, while Gly/Leu was only significantly different between samples defined as Pfirrmann grade III vs. Pfirrmann grades IV and V. The N-acetyl/α-Glc and N-acetyl/β-Glc ratios showed significant differences among all three Pfirrmann grades. Their values decreased with increasing levels of degeneration. Receiver operating characteristic (ROC) curve analysis identified N-acetyl/α-Glc = 3.28 as a good cut-off level for the best discrimination of Pfirrmann grades III and V, with a sensitivity of 83.3% and a specificity of 100%. The value of area under the ROC curve (AUC) = 0.92 confirmed that it is a very good tool for predicting increasing levels of degeneration (p = 0.033). It was also observed that the N-acetyl/β-Glc ratio, with a cut-off level of 2.27, allowed for discrimination of Pfirrmann grades III and V with a sensitivity of 100% and a specificity of 75% (AUC = 0.95, p = 0.027). These results are shown in Table 1.

4. Discussion

The better understanding of the structural and chemical basis of the MRI images made it possible to detect and define the early stages of intervertebral disc degeneration. Many classification systems have been proposed for lumbar disc degeneration [8]. In this study we use the Pfirrmann grading system, which was developed on the basis of the classification system described by Pearce et al. [9,10] and modified by Griffith et-al. [17]. Their studies showed that the changes in gross morphology, when the disc undergoes degeneration, were associated with specific alterations in MR images [11]. The biochemical analysis of the nucleus pulposus suggested that the changes in MRI signal intensity were not associated with the water content but with changes in proteoglycan concentrations [9,11,12].

![Fig. 2. Representative ex vivo 1H HR MAS NMR spectra of the degenerated disc with an increasing level of degeneration.](image-url)
The loss of MR signal was accompanied by decreasing proteoglycan content in the nucleus pulposus and increasing concentration of collagen [9,10].

In our study we compared concentrations of metabolites assessed by means of the HR MAS technique with the increasing level of disc degeneration determined on the basis of MRI images. The metabolic changes in degenerative disc disease were observed on the basis of the main components of intervertebral discs: proteoglycans and glycosaminoglycans. Degradation of the proteoglycans was observed in the 1H HR MAS spectra as an increase of amino acid levels [7,13].

Increases in concentrations of alanine, glycine, hydroxyproline, isoleucine, leucine and valine were correlated with an increasing level of degeneration (p < 0.05). The highest concentrations of these amino acids were observed in spectra with Pfirrmann grades IV and V (Fig. 2). More than a three-fold increase in the concentrations of hydroxyproline and glycine was associated with significant collagen breakdown occurring within the process of degradation. The concentration of hydroxyproline for Pfirrmann grade III was 0.49 ± 0.21 μmol/g, while for Pfirrmann grade V it was 1.79 ± 1.44 μmol/g. On the other hand, the concentration of glycine for Pfirrmann grade III was 0.47 ± 0.29 μmol/g, while for Pfirrmann grade V it was 1.48 ± 1.44 μmol/g.

Due to the decomposition of glycosaminoglycans (mainly chondroitin sulfate), the intensity of the N-acetyl peak in the proton spectra of the nucleus pulposus and annulus fibrosus decreased. In healthy disc tissues, the concentration of the N-acetyl peak was 8.7 ± 3.7 μmol/g, whereas in degenerated discs it decreased to 2.42 ± 0.69 μmol/g [7]. Moreover, significantly higher concentrations of α-Glc, β-Glc and myo-inositol were observed in discs with an increasing level of degeneration (p < 0.05). It was associated with the degradation of proteoglycans into their mobile constituents such as amino acids and sugars [5,7,15]. Myo-inositol is important for the regulation of cellular functions. It is an essential nutrient required for the growth and survival of human cells and an important osmolyte, which ensures osmotic equilibrium between the cells and the surrounding tissues [16]. In healthy disc tissue, concentrations of myo-inositol were 4.45 ± 1.31 μmol/g, whereas in degenerated discs it was found only in small amounts (1.46 ± 1.17 μmol/g) [7]. Changes in the concentration of MI may indicate an imbalance in osmolyte function of discs in degenerative diseases. We found different values between Pfirrmann grades III and V for the ratios of N-acetyl/β-Glc, N-acetyl/α-Glc, IPA/Ac, Gly/ Cr and Gly/Leu. This is the first study demonstrating that the N-acetyl/β-Glc, N-acetyl/α-Glc metabolite ratios determined by HR MAS NMR are potentially useful for accurate prediction of increasing levels of degeneration. The measure of quality of this method is the area under the ROC curve. Most of the tests used represent the diagnostic power for the values of AUC between 0.8 and 0.95. The high AUC values for the N-acetyl/β-Glc (AUC = 0.95) and N-acetyl/α-Glc (AUC = 0.92) metabolite ratios indicate high diagnostic power for discriminating levels of disc degeneration, especially for differentiating between Pfirrmann grades III and V. These metabolite ratios, as the main constituents of proteoglycan and glycosaminoglycan breakdown, may potentially serve as biomarkers for increasing levels of disc degeneration.

However, results of Fig. 3 indicate that (with the exception of hydroxyproline), concentrations of amino acids are higher in the Pfirrmann grade IV samples than Pfirrmann grade V. This finding may be due to the fact that Pfirrmann grade V is the highest grade of degeneration, when the amount of proteoglycans is so reduced that it influences the ratio of their mobile constituents they degrade into.

The significant relationship between the degree of degenerative changes and patient age is known [14,15]. In our previous study we observed that the concentrations of 2-propanol and lactate for lumbar (n = 55) and cervical (n = 24) discs increased significantly with increasing age in the nucleus pulposus and annulus fibrosus [7]. The concentrations of lactate and 2-propanol were increased in discs with a higher Pfirrmann grade, but this observation was not statistically significant.

![Fig. 3. Concentrations of metabolites in discs with different grades of degeneration assessed on the Pfirrmann scale.](image-url)
5. Conclusion

Our results provide another step toward the potential use of in vivo MR spectroscopy for the investigation of biomarkers in lumbar disc degeneration. We hope that in the near future HR MAS NMR ex vivo studies of metabolic profiles combined with in vivo studies using high-resolution MRI scanners (MRS) may become part of a new diagnostic protocol.

Acknowledgements

This work was supported by the Lodz University of Technology (Grant DS-I18/2013) and the Medical University of Lodz (Grant No. 502-03/5-138-06/502-54-068).

References