Influence of NMR Therapy on Metabolism of Osteosarcoma- and Chondrosarcoma Cell lines

B. Steinecker-Frohnwieser*, L. G. Weigl1, C. Höller2, E. Sipos3, W. Kulличich1, H. G. Kress3

© LBI for Rehabilitation of Internal Diseases, Ludwig Boltzmann Cluster for Rheumatology, Bioinformatics and Rehabilitation, Sausalito.
© Department of Special Anesthesia and Pain Management, Medical University Vienna, Austria.

INTRODUCTION

Magnetic resonance imaging (NMR) with weak magnetic fields (up to 2.3 mT, 100 Hz) has been shown to stimulate repair processes in cartilage and to influence pain signalling. Nowadays it has been proven that the therapeutic application of NMR has beneficial effects on different joint diseases and osteoarthritis. On the contrary, strong magnetic fields (2 T) used for imaging purposes are suspected to have deleterious effects on chondrocytes and cartilage repair.

The aim of our study was to demonstrate positive or negative effects of the therapeutic used NMR on the metabolism in chondro-, and osteosarcoma cells. The present investigations used NMR with weak field strength adapted to the specific types of tissue. Testing its influence on cellular processes we used micromass and quantitative PCR (qPCR) for expression profiling of NMR-treated chondrocytes and osteosarcoma cells. In addition, functional effects on Ca²⁺ influx and Ca²⁺ release were studied by applying of the Ca²⁺ imaging technique. Furthermore effects on protein functions involved in signal transduction pathways were studied using a luciferase reporter assay system.

METHODS

Cells

C2C12 cells were cultured in the presence of NMR stimulation (100 Hz, 2.3 mT for 20 h) into osteosarcoma cells and into chondrosarcoma cells. Cells used for RNA isolation were treated with an NMR therapy device (Desitec: MedTec, Wedel, Germany) for 20 h within 4 days (3 days 6 h each and 2 h on the last day). The extracted RNA was labelled with biotin and hybridized to membranes carrying different gene markers. The expression of specific genes was quantified by densitometry. In addition, quantitative PCR was performed to support the results from gene arrays. RNA of cells incubated at the same time at room temperature functional as control. Free Ca²⁺ concentration in living cells was determined fluorometrically with cells loaded with 2 μM for 5 min.

Chondrosarcoma cells were transfected with DNAs constructed corresponding to luciferase reporter genes to test for up and down regulation of the activation of regulatory proteins involved in signal transduction pathways.

IMPORTANT OSTEOLOGIE/REHUMATOLOGICAL FACTS

- Stress activated protein kinase (SAPK/JUN) is an enzyme of the MAPK family and are activated by a variety of environmental stress factors, inflammatory cytokines and growth factors.
- c-Jun signalling in combination with NFRF is crucial for RANKL-regulated osteoclast differentiation (Kida et al., 2004)
- NFRF has an important role within the transcription program of osteoclasts.
- NF-κB is activated by RANKL, and plays an essential role in osteoclastogenesis.
- The Fas Ligand (FasL) serves as a key death factor in the immune system by inducing apoptosis.
- The scaffold/MAPK/ERK pathway negatively regulates IgG stimulated PG synthesis in chondrocytes.
- Apoptosis inhibitor (IAP) family of proteins prevent cell death. The expression of BIRC3 (also called BIRC3) is increased following activity of NF-κB or TNF receptor.
- Glycogen synthase kinase 3 (GSK3-3) activity can dictate how cells will respond to glucocorticoids (Gallagher et al., 2006).
- Several growth factors (e.g. IGF, TGF, VEGF) regulate the behavior of cells in bone and cartilage and have a role in the inflammatory immune response.

CONCLUSION

At shown with the micromass technique, various genes are regulated to different extent compared to control.

- Obviously, the genes of the NFAT-pathway are regulated in a uniform manner.
- qPCR of the most noticeable genes didn't show significant changes compared to control.
- Luciferase reporter gene assays definitively showed any distinct increase but rather slight decrease of NF-κB or MAPK activity compared to untreated cells. This might be of importance because high levels of NF-κB and MAPK support the persistence of inflammatory processes in rheumatic diseases.
- Intraacellular Ca²⁺ signalling was not significantly changed due to the influence of NMR.
- From qPCR it can be concluded that NMR in therapeutic dosage does not induce apoptotic mechanisms in C2C12 and C4a-T8 cells.
- Arrays concerning apoptosis, cell cycle and osteogenesis are effective tools to evaluate responses to potential side effects of NMR therapy on osteosarcoma cells.

- Our results imply that NMR of a magnetic field strength up to 2.3 mT and therapeutic exposure time has no deleterious effects on osteo- and chondrosarcoma cells.

Fig. 1: In rheumatic diseases, certain signal cascades play a role in gene expression of factors with important functions in joint destruction, cell growth, apoptosis, inflammation, and immune response. These pathways can be included by stressors (e.g. UV radiation, mitogens, electromagnetic fields), by signals of growth factor receptors (e.g. TGF, EGF, IGFII) and cytokines (e.g. IL-6, INF-γ) as well as via Ca²⁺ signals of the NFAT pathway. Subsequently proto-oncogenes like ras, raf, etc. are upregulated in the cell, and various pathways are activated by kinases, for example the mitogen activated protein kinase (MAPK) signal pathway, the pathway of the nuclear factor of activated T-cells (NFAT). The E-1 receptor associated kinase (RAK) is generated via cytokine signals of NFAT and the information transmission factor NF-κB is activated. Thus, external signals are interlinked with internal signal pathways of the cell. In further consequence activation or expression of various genes can be initiated through transcription factors like c-jun, c-Fos, NFkB, NFAT, and gene promoters in the nucleus. In this way pathological processes in the joints can be regulated by chemokines, cytokines, RANKL, COX, metabolism, etc.

Fig. 2: Testing for long-term effects of NMR stimulation, different gene arrays (GeArray technology, Salfordiscience) were probed with RNA isolated from C2C12 and C4a-T8 cells treated by NMR for 20 h. Enrichment profiles were made up from pools of RNA in ideal concentration. For C2C12, duplicates of studied arrays were treated in two different experiments. The creatine kinase brain isoform type 2 (CKB) gene shows a very high signal with high intra- and interarray expression. Poutin the best cell NMR stimulation on C2C12 cells influences some but not all expression levels of gene worthards to be discussed in the context. The bars show the average gene values of two experiments—respectively a higher number in brackets—resulting from values of various gene arrays. Abundance and/or functions of analysed genes are given in the table.

Fig. 3: Possible NMR induced changes in gene expression identified by gene array technology were further studied by quantitative PCR performed with cDNA from isolated RNA of control and NMR treated cells. Figures plot changes assessed by qPCR in C2C12 cells (A) and in C4a-T8 cells (B) with respect to osteosarcoma cells. High amount of a specific cDNA profile was characterized by an early onset of fluorescence development. A twofold difference in cDNA concentration results in shift of one PCR cycle at a certain threshold value (CT value). For calculation of expression values, CT values of genes of interest were normalized to housekeeping genes (β-actin and GAPDH). Inserts represent the increase of fluorescence due to increasing concentrations of the amplicons.

Fig. 4: The time course of influence of NMR on signaling pathway activity was implemented by using the Cignal Finder® 10-Pathway Reporter Array (Salfordiscience), the ten reporter assays were tested interleaved (first table, column 1). C2C12 cells treated with different pathway focused transcription factor-responsive freely luciferase constructs were stimulated by NMR for three different time intervals (T). The figure further represents the averaged values out of four experiments for NMR treatment and three for control, the standard error is given in parenthesis. After the instant exposure C2C12 cells effectively transduced by positive control DNA based on a G-F construct. The summarized data for the second time point (T2) are presented by the bar chart.

Fig. 5: Time course of intraacellular Ca²⁺ concentration measured in A) chondrosarcoma cells, B) osteosarcoma cells. NMR treatment was before the measurement. Ca²⁺ release was triggered in C2C12 and C4a-T8 cells by application of histamine.

Quantification of Expression by Gene array

<table>
<thead>
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<th>Gene</th>
<th>Description</th>
<th>Log2 Fold Change</th>
<th>P-value</th>
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<tbody>
<tr>
<td>CKB</td>
<td>Creatine kinase brain isoform type 2</td>
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Quantification of Real-time PCR

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Quantification of Expression by Gene array

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