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NMR *In Vitro* Effects on Proliferation, Apoptosis, and Viability of Human Chondrocytes and Osteoblasts

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SUMMARY

This study presents findings on the proliferation rate, cellular apoptosis, and viability of human chondrocyte and osteoblast cultures before and after treatment with NMR pulse sequences. A commercially available nuclear magnetic resonance machine (MBST®—Nuclear Magnetic Resonance Therapy) was used for treatment. The study was carried out for 19 days, including 9 days of NMR exposure in a controlled, double-blind, randomized manner, using commercially available human cell lines. The study revealed that NMR treatment did not induce apoptosis or inhibit cell viability, but revealed a tendency of an elevated cell proliferation rate as observed by cell count. © 2005 Prous Science. All rights reserved.

Key words: Chondrocyte - Magnetic field - NMR - Osteoblast - Proliferation

INTRODUCTION

Magnetic field effects on cells have been studied earlier and its multiple effects have been published. However, technical as well as physical details of magnetic field applications (amplitudes, frequencies, application times, etc.) vary widely limiting somehow the validity of the observed data to the experimental conditions chosen (1–7). Little is known about nuclear magnetic resonance (NMR) effects on cells. NMR became popular in medicine as NMR imaging technology providing excellent transversal images of the human body. It was also used as a tool to elucidate analytical questions in modern medical research (8). Using NMR as potential tool for stimulating human cells has not yet been considered scientifically to an extent it might deserve in the future. When cells are placed in a strong magnetic field and a high frequency magnetic field pulse excites NMR, energy is deposited into the volume where resonance occurs. If this happens, one can expect that cellular metabolism might be affected and tissue growth as well as protein expression might be stimulated (9). Furthermore, signal transduction cascades might be activated (7, 10) and ion channels transport might be affected (11). These potential NMR effects on cells, however, lack a great deal of scientific approval.

Healings in human bone and cartilage tissue following MWR treatment have been observed in several clinical studies. Thousands of patients were treated with commercially available NMR stimulators (MBST®—Nuclear Magnetic Resonance Therapy). On the basis of these positive findings, we planned an *in vitro* study to evaluate the clinical results on the cell level. The study revealed that NMR treatment did not induce apoptosis or affect cell viability, but showed a tendency of an elevated cell proliferation rate quantified by cell count. These data encourage further *in vitro* studies on NMR–cell interactions that may make NMR treatments a scientifically based tool to regenerate tissue in the human body.

MATERIALS AND METHODS

Cell lines

For *in vitro* studies of NMR–cell interactions with MBST, cell lines of human chondrocytes and human osteoblasts were used (chondrocyte KIT-c (C-10710), osteoblast KIT-c (C-10720); Promocell, Heidelberg, Germany). Cells were cultivated in standard media and passage kits were suggested by the producers of the cell lines.

Experimental design

The study design was controlled, double blind and randomized. All investigations were carried out with the same NMR device. Chip cards carrying different programs for treating chondrocytes and osteoblasts, respectively, with NMR pulses (MedTech, Wetzlar, Germany) programmed the device. Chip cards for placebo treatments looked identical, but did not turn on the high frequency field necessary for NMR excitation.

The cell experiments were carried out at five different conditions (experimental groups):

Group 1—control, no static magnetic field, no high frequency field;
Group 2—static magnetic field, high frequency field for 30 min per day;
Group 3—static magnetic field, no high frequency field (placebo) 30 min per day;
Group 4—static magnetic field, high frequency field 60 min per day;
Group 5—static magnetic field, no high frequency field (placebo) 60 min per day.

The treatment was conducted over five subsequent days, followed by a 2-day pause and yet followed by another 4-day treatment. Afterwards, the cell cultures were cultivated for another 8 days without applying any magnetic field. The experiment was terminated on day 19. In all samples, the culture medium was changed every other day. At days 5 and 12, respectively, the cell cultures were split by factor 4.

Cell viability and apoptosis

Trypan Blue staining (VWR, Stockholm, Sweden) was used for determining cell viability (12). For determining the rate of apoptosis, the MitoCapture Kit (Merck Biosciences, Beeston, Nottingham, UK) was used. For positive apoptosis and for controls in cell viability experiments, cultures were incubated with H_2O_2 (13, 14) at 0.3 mM.

Proliferation rate

Cells were counted daily. Three random fields of identical size in the centre of the culture plates were imaged with a CCD camera mounted on a microscope. A software was developed allowing cell counting semi-automatically (Cell & Tissue Technology, Aachen, Germany).

RESULTS

The objective of the study was to determine whether MBST showed *in vitro* effects on apoptosis, viability, and proliferation of primary human chondrocyte and osteoblast cell cultures.

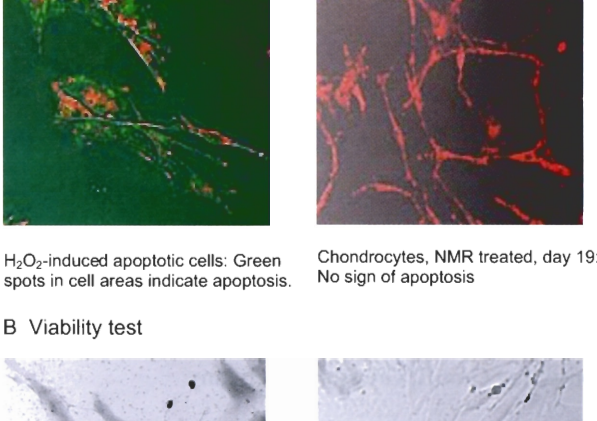
Apoptosis and viability tests

Except for positive controls, which were incubated with H_2O_2, all other groups did not show any sign of apoptosis or inhibition of viability. Figure 1 shows an example of images taken for apoptosis (Fig. 1A) as well as viability tests (Fig. 1B), respectively.

Proliferation rate

Table 1 shows the rates of proliferation for both cell types. Results of day 1 and day 15 (end of the treatment period), respectively, are displayed. The increase of the chondrocyte count was 271% cells over placebo, whereas the osteoblast count was 290% over placebo. The results showed positive tendencies on the proliferation after NMR treatment; however, no significant ones. This was mostly due to the low number of observations in this study (Figs. 2A and B). The obvious positive tendency of cell proliferation following NMR treatment

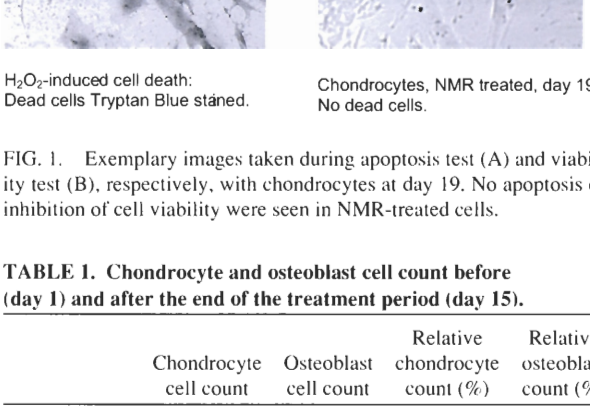
A Apoptosis test



H_2O_2-induced apoptotic cells: Green spots in cell areas indicate apoptosis.

Chondrocytes, NMR treated, day 19: No sign of apoptosis

B Viability test



H_2O_2-induced cell death: Dead cells Tryptan Blue stained.

Chondrocytes, NMR treated, day 19: No dead cells.

FIG. 1. Exemplary images taken during apoptosis test (A) and viability test (B), respectively, with chondrocytes at day 19. No apoptosis or inhibition of cell viability were seen in NMR-treated cells.

TABLE 1. Chondrocyte and osteoblast cell count before (day 1) and after the end of the treatment period (day 15).

	Chondrocyte cell count	Osteoblast cell count	Relative chondrocyte count (%)	Relative osteoblast count (%)
Day 1				
Verum	59	14	100	100
Placebo	69	17	100	100
Difference	10	3		
Day 15				
Verum	824	109	1397	779
Placebo	777	83	1126	488
Difference	47	26		
Difference (%)			+271	+290

Treatment was performed with MSBT(R)—Nuclear Magnetic Resonance Therapy fields. Verum, 30 min per day, static magnetic field with high frequency field at NMR frequency; placebo, 30 min static magnetic field, no high frequency field. After 15 days, the tendency of an enhanced proliferation following cell treatment at NMR conditions became visible: +271% for chondrocytes and +290% for osteoblasts, respectively.

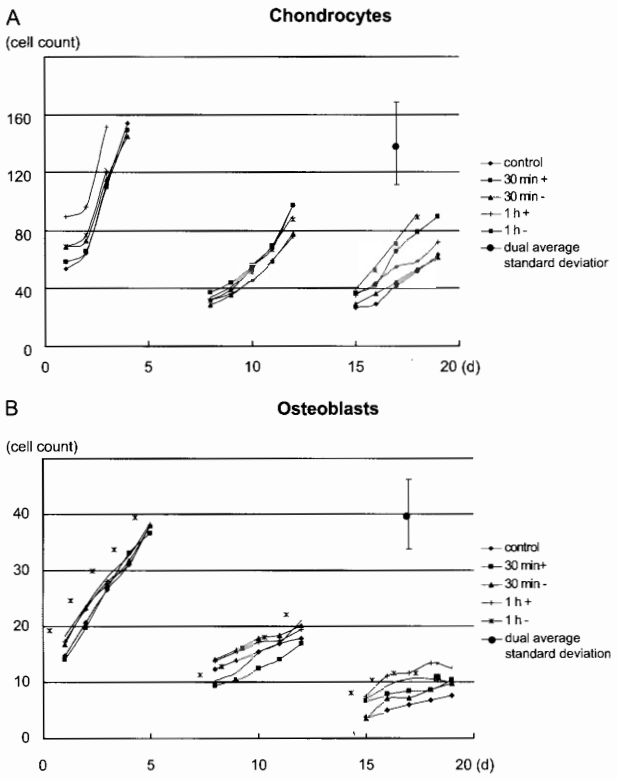


FIG. 2. (A) Chondrocyte proliferation. (B) Osteoblast proliferation. Since the cultures were passaged twice (1:4), there were three subsequent proliferation curves obtained for each group. The error bar up to the right shows the average dual standard deviation, including all cell count data of each individual sheet. Control: no static magnetic field, no high frequency field; 30 min +: static magnetic field with high frequency field (NMR resonance frequency) for 30 min per day; 30 min −: static magnetic field, no high frequency field; 1 h +: static magnetic field with high frequency field (MBST frequency) for 60 min per day: 1 h −: static magnetic field, no high frequency field.

(Verum) shown in Table 1 was expressed in very high percentages in their differences to placebo in both cell types.

DISCUSSION AND CONCLUSIONS

We observed in about 13,000 patients that MBST treatment healed numerous rheumatic joint disorders involving several international indices as WOMAC (15). These findings led to the idea that NMR could have an influence on the proliferation rate of osteoblasts and chondrocytes.

We used commercially available primary cell lines from humans. In this study, a positive tendency of cell proliferation due to NMR treatment was obvious (Table 1). It was found that NMR treatment *in vitro* neither induced apoptosis nor effected cell viability. This is most profound information, in particular, when NMR is applied to patients, since the results suggest that at the chosen NMR conditions negative effects in patients are most unlikely. This supports the widely accepted idea that the short-term (seconds to minutes) NMR application during clinical imaging is most likely not harmful (16). The investigation reported here was designed as a first step toward studying the biological mechanisms of bone and joint healing based on NMR. It was not designed to withstand profound statistic analysis. For proving statistical significance, much higher numbers of observations would have been necessary, which by far would have exceeded the frame and scope of our study. The results, however, suggest as conclusion that the NMR treatment based on MBST caused a visibly enhanced proliferation rate of primary cell culture of human chondrocytes and osteoblasts. At the same time, however, it became clear that NMR treatment did not induce apoptosis or inhibit viability of cells.

Despite the fact that in other studies (17-20), magnetic fields were used for cell stimulation and positive effects were found, those results cannot be simply adopted for NMR field stimulation without reconsideration. The physical nature of the magnetic fields applied (amplitude, frequency, time course of application etc.) plays a crucial role in stimulating cells. Thus, there is still a long road to go to understand NMR–cell interactions.

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