

# CYTOGENETIC EFFECTS OF MAGNETIC AND RADIOFREQUENCY FIELDS OF NMRI SYSTEMS ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN HUMAN LYMPHOCYTES IN THE PRESENCE OR ABSENCE OF CYTOSINE ARABINOSIDE

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## ABSTRACT

Application of nuclear magnetic resonance imaging (NMRI) as a non-invasive and accurate imaging procedure has been widely used in recent years. Meanwhile, the biological effects of magnetic fields of several tesla (T) and high energy radiofrequency (RF) is not fully known yet. Because of controversy over this issue, the present research has been carried out in order to verify the effects of magnetic fields of 1.5 T and RF of 63.86 MHz on the frequency of chromosomal aberrations in human peripheral lymphocytes.

Using metaphase analysis technique, the cytogenetic effects of NMRI was studied in G0 and G2 lymphocytes in the presence or absence of cytosine arabinoside (ara-C) as a DNA repair inhibitor. Cells were cultured using conventional methods.

Results obtained indicate that exposure of lymphocytes to NMRI field at 30 and 60 minutes has no potential effects on chromosomal aberration induction. When using ara-C, although ara-C alone caused a rather high frequency of chromosomal aberrations, especially in G2 phase of the cell cycle, exposure of cells to NMRI in the presence of ara-C did not change the frequency of ara-C-induced damage significantly.

Our results indicate that NMRI may not be able to produce DNA damage that could be potentiated by ara-C. Similar responses were also observed for cells exposed to NMRI either *in vivo* or *in vitro*. Nevertheless much remains unknown about the certain effects of MRI and RF.

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## INTRODUCTION

Study of biological effects of static magnetic fields is one of the most active yet controversial areas of investigation of the potential effects of clinical MR imaging, which

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is known as a powerful and non-invasive diagnostic tool.<sup>1</sup> While it is known that low doses of ionizing radiation are capable of causing cancer and genetic damage induction, this knowledge is lacking for magnetic fields associated with NMRI. Studies have been performed both *in vitro* and *in vivo* to demonstrate the presence or absence of chromosomal effects of static magnetic fields. Some of these studies have evaluated the biological effects of NMRI and RF based on chromosomal aberration induction, DNA damage and sister chromatid exchange (SCE), spermatogenesis, cell growth and survival of exposed tumor.<sup>2-6</sup> Experimental results suggest that electromagnetic fields can affect growth and DNA synthesis in many cell types.<sup>7-11</sup> Furthermore, other biological processes have been reported such as increase in cancer incidence.<sup>12-16</sup>

On the other hand, because carcinogenesis and mutagenesis are closely correlated,<sup>17</sup> and many mutagens are also considered as carcinogens, thus the initial damage induced by physical or chemical agents should be alterations in the structure of DNA, i.e. DNA damage. Determination of chromosomal aberrations which are the consequence of DNA strand breakage, is one of the most sensitive methods for detecting the effects of chemical and physical genotoxic carcinogens. The standard cytogenetic test, chromosomal aberration induction, has been proved to be an effective method for evaluation of physical mutagens such as ionizing radiation. Because of these controversies over the potential effects of NMRI and RF on the DNA molecule, we thought MRI might produce a kind of initial fast repair mechanism of DNA damage which can not be detected as chromosomal aberrations in mitosis. In this study we used cytosine arabinoside, a DNA repair inhibitor<sup>18,19</sup> which potentially prevents repair of X-ray induced DNA damage, thus leading to an increased level of chromosomal aberrations<sup>20-22</sup> as well as to prevent repair of probable DNA damage induced by NMRI.

## MATERIALS AND METHODS

### Culture of unstimulated lymphocytes

Human venous blood samples were taken with a heparinized syringe from four young healthy non-smoker male donors (age  $30 \pm 4$  years). For all experiments 0.4 mL whole blood was mixed with 4.5 mL RPMI 1640 medium (Sigma) supplemented with 15% heat inactivated fetal calf serum (Flow) and antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) (Sigma) in a closed tube. Ara-C at 50 and 100 µmol/L was added to eight cultures (4 for each concentration) and were incubated at 37°C in a dry incubator for three hours prior to magnetic and RF exposure. After 60 minutes exposure of the sample to NMRI and RF fields in the presence or absence of ara-C, cells were washed twice in fresh RPMI medium and deoxycytidine (Pharmacia, LKB) was added twice as the concentration of ara-C to culture

vessels in order to reverse ara-C effects on cells. Once treatment was completed, 0.2 mL of phytohemagglutinine (PHA-M) was added to all cultures. They were incubated at 37°C for 52 h when they were fixed. Colchicine (Sigma) at a final concentration of 4 µg/mL was added to each culture two hours prior to fixation. Fixation and slide preparation was performed according to conventional procedures and nonbanded, Giemsa stained chromosome preparations were made.

### Culture of stimulated lymphocytes

Lymphocyte cultures from heparinized whole blood were set up as for unstimulated cultures, except that PHA was added immediately to the culture medium. The cultures then were incubated at 37°C for 48h, then ara-C was added to 8 cultures at 50 µmol/L and 8 other cultures at 100 µmol/L concentration 1h prior to combined NMRI and RF exposure. Ara-C was present during and after exposure until fixation. Colchicine at a final concentration of 4 µg/mL was added immediately after exposure, so it was present for 2h. Fixation and slide preparation was performed according to the conventional procedure. Two hundred to four hundred metaphases were analysed for each sample and aberrations were classified as chromosome type aberrations (breaks, dicentric and rings) for unstimulated lymphocytes and chromatid type aberrations (deletion and exchanges) for stimulated lymphocytes in G2 phase. Mitotic indices were obtained by counting 3000 cells per each sample under 40× objective magnification. The significance of any intergroup differences in the number of chromosome aberrations were statistically evaluated by one way analysis of variance (ANOVA) and Student's t-test.

### Exposure to a magnetic field and radiofrequency waves

Each sample was placed in a separate plastic tube exposed to a magnetic field of 1.5T alone or in the presence of ara-C using MRI equipment (Vista, Picker), and to radiofrequency waves (63.86 MHz, specific absorption rate, SAR 0.4 W/kg). Samples were placed in the center of the magnetic field and the combined effect of MRI and RF was studied for 30 min and 60 min respectively under exposure protocol of T1 weighted and spin echo technique with a repetition (TR) of 600 msec and time echo (TE) of 20 msec.

### *In vivo* effects of NMRI on G0 lymphocytes

In order to compare the effects of NMRI on human peripheral lymphocytes *in vivo* and *in vitro*, blood samples were obtained from 6 male patients admitted to a spine imaging cage just before and after NMRI exposure. Patients were exposed for 30 minutes in an identical condition. Lymphocytes were cultured as described above for unstimulated lymphocytes and 100 mitoses were analysed for each sample.

**Table I.** Frequency of chromosome type aberrations in G0 lymphocytes following exposure to NMRI in the presence or absence of ara-C.

Treatment	No. of cells analysed*	Chromosome aberrations			Mean aberrations/100 cells $\pm$ SE
		Isogaps	Deletions	Exchanges	
<i>In Vivo:**</i>					
Samples before NMRI exposure	600	1	2	1	0.66 $\pm$ 0.33
Samples after NMRI exposure	600	2	1	1	0.66 $\pm$ 0.33
<i>In Vitro:</i>					
Control	200	1	0	0	0.5 $\pm$ 0.5
NMRI 60 min.	200	0	1	0	0.5 $\pm$ 0.5
Ara-C 50 $\mu$ mol/L	200	0	1	3 dic.	2.0 $\pm$ 1
Ara-C 50 $\mu$ mol/L+ NMRI 60 min.	200	0	1	3 dic.	2.0 $\pm$ 1
Ara-C 100 $\mu$ mol/L	200	0	0	4 dic.+ 1 ring	2.5 $\pm$ 1.1
Ara-C 100 $\mu$ mol/L+ NMRI 60 min	200	0	0	3 dic. + 2 rings	2.5 $\pm$ 1.1

\*Pooled data of two independent experiments.

Errors are standard errors of mean values.

\*\*Pooled data from 6 different patients admitted for NMR imaging just prior to or just after exposure to NMRI.

## RESULTS

### Study of G0 lymphocytes

As shown in Table I, exposure of G0 lymphocytes to NMRI for 60 minutes exposure time-two times that used in routine clinical practice did not increase the frequency of chromosomal aberrations above the control level. On the other hand some samples were treated with ara-C three hours prior to exposure with NMRI and then underwent exposure. Although ara-C alone causes chromosomal aberrations in unstimulated lymphocytes in low frequency, the frequency was significantly low to not be considered as an increase in aberration.<sup>20</sup> Indeed exposure of NMRI in the presence of 50 and 100  $\mu$ mol/L ara-C did not enhance the yield of chromosomal aberrations and was not statistically significant ( $p=0.351$ ) (Table I).

### Study of G2 lymphocytes

Results are shown in Table II. Exposure of G2 lympho-

cytes to NMRI for 30 and 60 minutes did not produce any chromosomal aberrations above the control level. Ara-C alone at 50  $\mu$ mol/L significantly increased the chromatid type aberrations as compared with control groups ( $p<0.05$ ). Chromosomal aberrations induced by ara-C alone at 100  $\mu$ mol/L was higher than 50  $\mu$ mol/L (16.25 versus 12.25 per 100 cells). This indicates the clastogenic effects of ara-C on lymphocytes at the G2 phase of the cell cycle. Exposure of G2 cells to NMRI for 30 and 60 minutes in the presence of 50  $\mu$ mol/L ara-C did not increase the frequency of chromosomal aberrations induced by ara-C alone ( $p=0.337$ ). Similar results were obtained for 100  $\mu$ mol/L ara-C treated cells after exposure to NMRI for 30 and 60 minutes. Our results also indicate that there was no significant difference between the frequency of induced chromosomal aberrations by ara-C alone and those samples exposed to NMRI in the presence of ara-C ( $p=0.7836$ ). Although mitotic indices scored for ara-C treated samples and exposed to NMRI in the presence of ara-C are significantly different ( $p<0.05$ ), exposure

**Table II.** Frequency of chromatid aberrations induced in G2 phase of the cell cycle following exposure to NMRI in the presence or absence of ara-C.

Treatment	No. of cells	Chromatid Aberrations			Mean aberrations/ 100 cells±SE	Mean Mitotic Index/ 1000 cells±SE
		tGaps	Deletions	Exchanges		
Control (Untreated)	400	0	0	2	0.5±0.35	2.3±0.54
NMRI 30 minutes	200	0	0	1	0.5±0.5	3.1±0.49
NMRI 60 minutes	200	0	0	1	0.5±0.5	3.23±0.35
Ara-C 50 µmol/L	400	0	38	11	12.5±1.75	0.98±0.33
Ara-C 50 µmol/L+						
NMRI 30 min.	200	0	22	3	12.5±2.5	1.92±0.7
Ara-C 50 µmol/L+						
NMRI 60 min.	200	0	16	5	10.5±2.29	2.6±0.1
Ara-C 100 µmol/L	400	0	59	18	19.25±2.19	0.57±0.16
Ara-C 100 µmol/L+						
NMRI 30 min.	200	0	26	11	18.5±3.04	1.27±0.22
Ara-C 100 µmol/L+						
NMRI 60 min.	200	0	30	9	19.5±3.12	2.4±0.4

\*Pooled data of 2 independent experiments.

Errors are standard errors of mean values.

of cells to NMRI led to an increase in mitotic index (Table II).

#### *In vivo* exposure of G0 lymphocytes

No significant differences in chromosomal aberrations were observed in lymphocytes of patients before and after exposure to NMRI ( $p=0.791$ ) (Table I).

### DISCUSSION

Exposure to NMRI for 30 and 60 minutes did not affect G0 and G2 cells and the chromosomal aberrations observed were not statistically significant in comparison with the control group. Increase of NMRI exposure time, about two folds that in routine practice (60 minutes) did not produce chromosomal aberrations above the control level. These findings are consistent with many previously reported results.<sup>2,3,6,23-26</sup> However, in another study reported by Khalil and Qasem (1991), an increased frequency of chromosomal aberrations and cell death was observed following pulsed electromagnetic field exposure in human lymphocytes.<sup>27</sup> Study of human lymphocytes from patients exposed to NMRI for 30 minutes clearly shows that the cell response to NMRI is identical both *in vivo* and *in vitro*. When G0 lymphocytes were treated with ara-C alone, a significant number of chromosomal aberrations was not produced (Table I) as was reported by Preston.<sup>20,29</sup> This result is inconsistent with the observation of Pantelias and Wolff for G0 lympho-

cytes.<sup>28</sup> Ara-C alone at 50 and 100 µmol/L effectively caused a high frequency of chromatid breaks and gaps but not exchanges (Table II). Preston<sup>20</sup> and Natarajan et al.<sup>30</sup> showed that inhibitors such as hydroxyurea and ara-C significantly reduce the frequency of exchange type aberrations in G2 cells by inhibition of the rejoining process of DNA strand breaks.

Following exposure of G0 and G2 cells to NMRI in the presence of ara-C, the yield of chromosomal aberrations did not differ with ara-C treated cells (Table I and II). It is shown that ara-C is able to increase the frequency of chromosomal aberrations induced by other physical agents such as X-rays either by the incision of DNA at sites of base damage<sup>20,21,31</sup> or by the inhibition of repair of both single and double strand breaks.<sup>30</sup> If this is the case, then we may conclude that NMRI exposure at 1.5 T in routine clinical use might not produce such DNA damage in lymphocytes to be converted to chromosomal aberrations by cytosine arabinoside, at least in the first generation of cells. Because of controversial epidemiological or experimental findings, much remains unknown about the certain effects of MRI and RF.

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