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Electric and magnetic noise blocks the 60 Hz magnetic field enhancement of steady state *c-myc* transcript levels in human leukemia cells

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Abstract

We have previously demonstrated that exposure of human cultured cells to 60 Hz sinusoidal magnetic fields causes an increase in steady state transcript levels for the proto-oncogene *c-myc*. A study by Litovitz et al. (T.A. Litovitz D.Krause and J.M. Mullins, Biochem. Biophys. Res. Commun., 178(1991) 862) suggests that the induction of bioeffects from exposure to 60 Hz fields requires that the exposure field be coherent for some minimum length of time (approximately 10s). A related study demonstrated that these effects can be blocked by superposition of temporally incoherent magnetic noise fields. In this paper we investigate whether or not the superposition of a magnetic noise field can block the bioeffect of a coherent 60 Hz magnetic field on the transcript levels of the proto-oncogene *c-myc* in HL60 cells. Experiments were performed using band-limited magnetic noise fields (30–90 Hz, 6.7 μT rms), and coherent 60 Hz magnetic fields (6.7 μT rms). The results show that exposure to the 60 Hz field alone produces approximately a 40% enhancement in the steady state level of the *c-myc* transcript. Superposition of the noise field on the 60 Hz field inhibited the enhancement of the *c-myc* transcript to the extent that no statistically significant was observed. This study provides additional proof of the coherence time requirement, and of the validity of the noise superposition method to block electric and magnetic field induced bioeffects.

Keywords: Transcript; *c-myc*; Electric and magnetic field; Noise

1. Introduction

Biological effects from exposure to extremely low frequency (ELF) electric and magnetic (EM) fields have been demonstrated in numerous studies. While the occurrence of bioeffects does not necessarily imply an imminent biohazard, a number of epidemiologic studies have indicated that there is a potential link between the incidence of certain types of cancer (in particular childhood leukemia) and exposure to weak low frequency EM fields [1–4]. The possibility of a cancer connection has stimulated studies at the cellular level, including some of our previous work, on the effects of 60 Hz EMF exposure on biological end points relevant to cancer promotion such as the expression of proto-oncogenes [5–9]. We previously showed that exposure of HL60 cells to weak 60 Hz sinusoidal electric and magnetic fields results in changes in the steady state level of some transcript. The affected genes include proto-oncogenes, transcription factors and housekeeping genes. One of these genes is the proto-oncogene *c-myc*. This gene is important because on mutation, it can become an oncogene.

The mechanisms by which electromagnetic fields affect gene transcription and other biological functions are not fully understood. A clue to these mechanisms has been provided by experiments in which it was demonstrated that the enhancement of onithine decarboxylase (ODC) activity in L929 cells induced by exposure to a 60 Hz magnetic field required that the exposure field be coherent for a minimum length of

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time, approximately 10 s [10]. Based on these findings, Litovitz suggested that the EMF induced ODC enhancement in L929 cells in particular, and other electromagnetic field effects in general, could be inhibited by any means which made the exposure signal incoherent, such as superposition of electromagnetic noise. This hypothesis was tested in two separate experiments measuring different biological end points. The first demonstrated that 60 Hz magnetic field induced ODC enhancement in L929 murine cells can be blocked by simultaneous application of an ELF noise EM field [11]. The second showed that abnormalities induced in chick embryos by application of an ELF magnetic field can be eliminated by superposition of an ELF noise EM field [12,13]. The purpose of the present study was to investigate whether or not this technique, that is superposition of a noise EM field, can also be used effectively to block changes in *c-myc* transcript levels induced in human leukemia cells (HL60) by exposure to coherent magnetic fields.

2. Materials and Methods

2.1. Cell preparation

Human leukemia cells (HL60) in growth phase were cultured in T-175 flasks and subcultured into T-25 flasks at an equivalent cell density of 1×10^6 cells ml in 15 ml culture medium. A set of four samples in T-25 flasks was prepared from a single flask for each experimental sequence 3 h before each run. The cells were maintained and exposed in RPMI 1640 with L-glutamine (GIBCO) and 10% fetal calf serum. All exposures were conducted for 20 min at 37.5°C.

2.2. Exposure apparatus

Cell exposures were carried out using two pairs of Helmholtz coils (Electro-Biology Inc., EBI) arranged such that one pair (inner coil pair) was placed within the other (outer coil pair). Both coil pairs were mounted vertically so as to produce parallel and horizontally oriented magnetic fields. Sample flasks were exposed individually within the coils, positioning each flask such that its broad base was oriented parallel to the direction of the magnetic field. With this configuration, electric fields are induced within the culture medium on planes perpendicular to the direction of the magnetic field. The magnitude of the induced field at the base of the flask, where suspended cells tend to settle, varies approximately in direct proportion to the depth of the culture medium. For small depths, such as in this case (0.6 cm), the induced field is constant to within 8% of the maximum over 85% of the base. The maximum induced electric field at the base, for a depth of 0.6 cm, is approximately 7.6 μV m^{-1} for a 6.7 μT rms exposure field [14,15].

Both Helmholtz coils were made using 164 turn rectangular windings of gage 19 magnet wire measuring approximately 13 cm × 14 cm. Support was provided by a Plexiglass frame. The total resistance of each Helmholtz coil pair was 3.7 Ω. The coil separation of the inner coil pair was approximately 8 cm, and that of the outer pair approximately 11 cm. Magnetic shielding was provided by placing the dual Helmholtz exposure apparatus within a closed cylindrical μ-metal enclosure measuring 30.5 cm in diameter by 30.5 cm in height. Magnetic field measurements inside and outside the μ-metal enclosure were made using a calibrated search coil and integrator to determine possible leakage into or out of the enclosure. With the exposure field off, the measured background field within the μ-metal enclosure was below the limit of detection of the search coil (1 mG). Similarly, with an exposure field of 6.7 μT rms, the exposure level used in these experiments, the leakage field measured outside the μ-metal enclosure was also below the limit of detection of the search coil. Temperature control was achieved by placing the magnetically shielded exposure apparatus within an incubator maintained at 37.5°C. Temperature measurements were taken using a thermocouple with 0.1°C resolution (Physitemp Inc.). Control samples were run concurrently with exposed samples within a 30.5 cm × 30.5 cm × 28 cm μ-metal box located below the exposure apparatus within the same incubator in sham coils.

60 Hz sinusoidal magnetic fields were imposed using the inner Helmholtz coil pair of the exposure apparatus. These coils were driven with a model 21 Wavetek function generator connected to a three position power splitter supplied by the coil manufacturer (Electro-Biology Inc.). The power splitter was adjusted to a setting producing a 6.7 μT rms magnetic field within the Helmholtz coil. This field was measured with an IDR-90 60 Hz magnetic field meter (Integrity Electronics) with the exposure apparatus inside its μ-metal enclosure. Band-limited noise magnetic fields (30–100 Hz) were superimposed over the coherent fields using the outer Helmholtz coil pair of the exposure apparatus. These coils were driven with a custom made band-limited noise source connected to 35 W audio amplifier (Realistic, model 35, Tandy Corp.). The noise amplitude was adjusted such that the average noise fluctuations, viewed on an oscilloscope displaying the integrated output of a pick-up coil, were of the same order of magnitude as the oscillations of the 60 Hz magnetic field. This noise field level was selected based on previous results which show that full inhibition of magnetic field effects can be achieved when the rms value of the superimposed noise field is equal to the rms value of the ELF coherent field [12,13]. Three expo-

sure conditions were examined; these are summarized in Table 1.

2.3. Exposure procedure

Each field exposure condition (1 and 2, Table 1) was run for 20 min intervals concurrently with a control sample. Five sets of samples were tested using this procedure.

2.4. RNA extraction

After each exposure the flasks were immersed in an ice slurry for 10 min. All subsequent steps were carried out at 4°C. The cells were pelleted by centrifugation in a clinical centrifuge for 5 min followed by removal of the supernatant. The cells were then lysed by suspending the pellet in lysing buffer [5,8] and mixing with a vortex at 15 s intervals for 2 min. RNA extraction was accomplished as previously described [5,8]. The integrity of the RNA was checked by electrophoresis on a 1% agarose gel, identifying the 5S, 18S, and 28S bands of ribosomal RNA following ethidium bromide staining of the gel [16].

2.5. Measurement of c-myc transcript

Quantitative dot blot hybridizations were used to measure endogenous quantities of *c-myc* transcript [17]. Northern blots were used to confirm that only *c-myc* transcript was hybridized, as well as to confirm the size of transcript. Dot blot hybridizations were performed using nitrocellulose filters. Nytran filters were used for Northern blots. The *c-myc* DNA (Oncor) was labelled *in vitro* with ^{32}P dCTP (specific activity approximately 10×10^7 cpm μg^{-1}) [16,17].

Each dot blot series was performed with three 1:1 dilution steps starting with 3 μg total RNA. The extent of *c-myc* binding by the probe on each blot was quantified by radioactive measurements. The blotted regions of the nitrocellulose filter identified by the autoradiographic spots were cut out and counted in a liquid scintillation counter. The background counts were determined by counting a non-radioactive region of the nitrocellulose filter. In some cases, RNA samples were probed for $\beta 2$ microglobulin transcripts as an internal control [18]. Transcript levels for this gene are unaffected by TPA or EM field exposure [7].

Table 1
Exposure conditions

Exposure condition	60 Hz (μT rms)	Noise (μT rms)	Exposure time (min)
1. Coherent signal	6.7	0	20
2. Coherent signal + incoherent signal	6.7	≈ 6.7	20
3. Control	0	0	20

Table 2
Summary of results of experiments comparing exposed samples to control samples; each entry was calculated as the ratio of the slope of the activity vs. concentration curve of the exposed sample to that of the control sample

Experiment	60 Hz	60 Hz + noise
1	1.41	0.91
2	1.45	0.97
3	1.35	0.87
4	1.22	0.89
5	1.41	1.18
Mean	1.37 ± 0.08	0.097 ± 0.11

3. Results

Table 2 summarizes the results of each set of runs. Each entry was calculated as the ratio of the slope of the radioactivity (counts per second) vs. concentration (micrograms total RNA) curve of the exposed sample to that of the control sample. Theoretically this curve should yield a straight line with a zero intercept. However, in practice this is difficult to achieve because of confounding factors such as non-linearities of the counting equipment, and background radiation. Both the intercept and the slope of each curve were computed. The slope, which is a measure of the binding of the *c-myc* probe per micrograms total RNA, was used for comparison of exposed and control samples. The tabulated ratios of the slopes are a measure of the fractional change in *c-myc* expression of exposed samples relative to controls.

From the tabulated results it is apparent that exposure of HL-60 cells to a 60 Hz sinusiodal field for 20 min produces an increase in the expression of the *c-myc* gene relative to control cells. Likewise, it is also apparent that the effect of the 60 Hz field on *c-myc* expression is inhibited by superposition of the incoherent band-limited noise field. The results were examined using a two-tailed *t*-test to test the hypothesis that the ratio of the exposed samples over the control samples was equal to unity. The statistical test shows that the average ratio of exposed to control samples for the 60 Hz exposures is statistically different than unity with $p = 0.05$, indicating a positive effect. By contrast, the average ratio of exposed to control samples for the 60 Hz + noise data is shown to be statistically equal to unity with $p = 0.05$, indicating no statistically significant effect. The observed increase in *c-myc* expression from exposure of HL-60 cells to 60 Hz fields is of the order of 40%, which is similar to previous results.

Superposition of a noise field oriented parallel to the 60 Hz field clearly inhibits the effect of the 60 Hz magnetic field.

4. Discussion

The basic result from this research is that exposing HL60 leukemia cells to a 60 Hz magnetic field induces a significant increase in the steady state transcript levels of *c-myc* and that this increase can be inhibited by the simultaneous application of an EM noise field. This result is similar to that described by Litovitz et al. in L929 murine cells [13].

In an earlier report Litovitz et al. [10] demonstrated that unless the impressed sinusoidal signal exhibits temporal coherence for time intervals on the order of 5–10 s, no increase in ODC activity is produced. The applied noise fields in the experiment reported here are temporally incoherent for time scales longer than about 0.02 s (this number is related to the bandwidth of the noise); they fail to satisfy the temporal coherence criterion and are therefore incapable of stimulating a bioresponse. Moreover, the superposition of an incoherent field on the coherent 60 Hz field yields a field that is incoherent, the degree of the incoherence being dependent on the relative amplitudes of the incoherent (noise) and coherent components. Litovitz *et al.* reported that when the rms value of the noise field was equal to that of the coherent 60 Hz field, complete suppression of the EM induced effect occurs. These are the conditions used in the experiments reported here.

The mitigation of the magnetic field induced bioeffect by the simultaneous application of a weak noise field is at first puzzling when one considers that cells exist in an environment that is naturally abundant with electromagnetic noise. The random thermal motion of ions in the vicinity of cells leads to the presence of fluctuating fields that are roughly 1000 times larger than the externally imposed sinusoidal field and noise fields used in this experiment roughly 0.1 mV/cm^{-1} for the rms endogenous thermal noise field compared with approximately 0.1 $\mu V/cm^{-1}$ for the amplitude of the induced electric field component of an externally imposed 60 Hz, 10 μT magnetic field [19]. The cellular effect of the sinusoidal field is blocked by the weak external noise field, but is undisturbed by the much larger thermal noise field. Cells have apparently evolved in such a way that the ever-present, random, electromagnetic background does not affect cell function. The data presented here suggest that cells can distinguish between exogenously applied noise and the ever-present endogenous thermal noise. Some fundamental differences in the properties of these two noise fields must provide the basis for such differentiation. Litovitz and coworkers [12,13] have suggested that although both noise fields are (by definition) temporally incoherent, they exhibit a distinct difference in their spatial behavior. At a given time, the magnitude and direction of the thermal noise field at any point is uncorrelated with its value at other locations more than a few nanometers or so distant. This can be explained using the Debye screening length (roughly the range over which an ion is not shielded from other ions) which in the extracellular fluid is only about 1 nm. Thermally driven localized charge density fluctuations, and consequently endogenous thermal noise fields, are spatially incoherent over distances greater than a few nanometers. By spatially incoherent we mean that the amplitudes and phases of the endogenous fields at any point on the cell surface are random with respect to any other point greater than a few nanometers away. By contrast, exogenously impressed fields always exhibit spatial coherence since the phase and amplitude of the applied field is the same at all points on the cell. Litovitz and coworkers [12,13] hypothesized that it is the spatial incoherence of the thermal noise field that keeps the cells from responding to it. The cells do sense all externally applied fields because they are spatially coherent, but modification of cell function occurs only when these fields are temporally coherent. The coherence time of these exogenous fields must be approximately 10 s or greater.

If spatial coherence is the critical field characteristic that enables cells to respond to exogenous EM fields, then it can be inferred that the biological targets of electromagnetic fields are spatially extended or distributed. Two possibilities are that (1) there is a collection of receptors on each cell that must be coincidentally excited to produce the observed biochemical responses, or (2) a rather large number of cells must be simultaneously stimulated. In either case some cooperativity among the receptors involving intracellular or intercellular signaling, respectively, must be operative.

Litovitz et al. [12] proposed a possible explanation of the first of these two possibilities. Cellular detection of EM fields is assumed to result from the impressed fields modifying the binding of ligands at their receptor proteins, resulting in a change in cellular bioynthesis. The requirement that many receptors be activated simultaneously—*cooperativity*—prevents random activation of individual receptors and thus the triggering of an erroneous cellular response. This leads to the requirement of spatial coherence in the EM field if a cellular response is to be evoked. In this model, thermal noise fields, which are spatially incoherent, are incapable of causing any modification in cellular behavior.

An alternative explanation involving spatial coherence of the field is the supposition that communication and cooperation among cells via gap junctions is required for there to be an alteration of cell functioning

[19]. Since HL60 cells do not, however, form gap junctions, it appears that this explanation cannot be applied to our data.

5. Conclusions

The results of the present study support the hypothesis that the cell requires a stimulating EM field with a certain minimum degree of time coherence in order to respond to the field. In addition it appears that a certain degree of spatial coherence is needed. Furthermore, this study provides additional proof of the validity of the noise superposition method proposed by Litovitz as a technique for blocking EM field induced bioeffects.

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