Effect of ELF MF on Gap Junction Intercellular Communication, Its Mechanism and Interference

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Growth of normal cells is dependent, to some extent, on their ability to recognize and to communicate with neighboring cells. Gap junctional intercellular communication is an important kind of exchange among adjacent cells in living systems, and plays an essential role in regulation of cell growth, differentiation, and proliferation. Many experiments support the idea that disruption of GJIC may be a significant factor in cancer promotion. A series of experiments concerning the effects of 50 Hz magnetic fields on GJIC were conducted in our lab to explore if the ELF MFs may act as cancer promoter or be synergistic with TPA in cancer promotion, and to explore its mechanism and interference.

1. Effects of ELF MF on GLIC

The GJIC of cells was determined by dye transfer assay with two different methods. One was microinjection. The number of dye-coupled cells (DCC) per injection of the dye was used as an index of GJIC. The other was fluorescence-recovery-after-photobleaching (FRAP) analysis, and the percentage of fluorescence recovery was used as the index of GJIC.

With the method of microinjection, the effects of 50 Hz MFs at different intensities with/without TPA (5ng/ml) on GJIC in Chinese hamster lung (CHL) cells were studied. The results showed that the suppression of GJIC due to magnetic field exposure was related to the field strength. The disruption of GJIC induced by MF at 0.8 mT occurred not only in the group exposed to MF combined with TPA, but also in the group exposed to MF alone; and the effect of field exposure was very similar to 5 ng/ml TPA treatment in inhibition of GJIC in CHL cells. Although MF treatment alone at lower intensities (0.2 and 0.4 mT) did not affect GJIC, these MFs could enhance the suppression of GJIC by TPA. When the magnetic flux density was as low as 0.05 mT, no effect on GJIC was observed.

The inhibition effects of 50 Hz MFs on GJIC in CHL cells were demonstrated by FRAP analysis with a laser-scanning conforcal microscope The FRAP analysis for measuring GJIC is more sensitive than the method of microinjection. The threshold level of 50 Hz MF for GJIC inhibition showed that 0.4mT MF exposure alone may significantly suppress GJIC, which is lower than the occupational exposure standard proposed by ICNIRP. Similar results were also observed in NIH3T3 cells.

Pulsed ELF MFs (PMF) have been successfully used for patients with ununited fractures and for wound healing. It was supposed that the PMF might suppress GJIC to benefit the cell proliferation as one of the important mechanisms for wound healing

by PMF. The effects of a pulse MF with 50 Hz repetition frequency, 2ms pulse width on GJIC in CHL cells were studied with the method of microinjection. The results showed that the cells exposed to the PMF at 0.4mT or 0.8mT for 24 h were significantly suppressed in their GJIC and there were significant differences in DCC when compared to sinusoidal MF with the same average flux density, indicating that the PMF is more effective than the sinusoidal MF in down-regulation of GJIC.

2. The Mechanism of GJIC Suppression by ELF MFs

Gap junctions can be upregulated or downregulated at the transcriptional, translational, and posttranslational levels by endogenous or exogenous factors.To explore the molecular mechanisms, the effect of 50 Hz, 0.8-mT MF on connexin 43 gene transcripion was studied. Transcriptional levels of the connexin 43 gene in the mouse fibroblast cell line NIH3T3 and CHL cells were examined by Northern blot analysis using a ^{32}P-labeled connexin43 probe. Cells had been exposed to MF for 24 h and /or had received 2h of TPA treatment. The connexin43 gene transcriptional level did not change significantly in any exposure groups. The loss of GJIC is therefore not due to an effect of MFs and/or TPA at the transcriptional level.

Protein kinase C (PKC) is known to phosphorylate connexins. We suppose that PKC may be involved in the suppression of GJIC by MF. An experiment with the PKC inhibitor staurosporine (STS) or palmitoyl carnitine (PMC) was carried out. Chinese hamster lung (CHL) cells were exposed to 50 Hz at an average flux density of 0.8mT for 24 h. They were treated with PKC inhibitor (STS or PMC) at different concentrations during the last hour. The results showed that the suppression of GJIC induced by PMF was dramatically inhibited in the presence of STS or PMC. We concluded that the inhibition of GJIC induced by ELF MF may be due mainly to hyperphosphorylation of gap junctional connexins.

The phosphorylation status of gap junction proteins plays an important role in the gating of gap junction channels. We investigated the effects of ELF magnetic field on protein levels and phosphorylation of connexin 43 (Cx43) with Western blot analysis. The results showed that ELF and/or TPA exposure induced a relative decrease in P_0. and P_3 was detectable after the treatment. In addition, It was showed that Cx43 protein level did not appear to be substantially altered by any of the treatments.

We then studied the effect of ELF MF on the localization of Cx43. CHL cells were exposed to 0.8mT, 50Hz MF for 24h and /or 5 ng/ml TPA for 1 h. The results showed that many gap junction plaques were bright labeling at regions of intercellular contact in control cells. The treated cells, either with ELF MF or with TPA, displayed an entirely different pattern: with less and punctuate labeling, and a large part of them appear in the cytoplasm and nuclear area of the cells. Internalization of Cx43 proteins was observed in exposed cells.

Taking these data together, it can be concluded that the molecular mechanisms of suppression GJIC by ELF MF is at the post translation level. The inhibition of GJIC induced by ELF MF is due primarily to hyperphosphorylation of gap junctional connexins mediated by PKC, and the internalization of Cx43 from plasma membrane

to cytoplasm and nuclear.

3. Interference of ELF MF induced GJIC suppression by MF noise

Litovitz reported that the presence of MF noise, comparable in magnitude to the MF signal, could nullify the MF biological effects. We studied the effect of superposition of a 0.4mT MF noise(30 –100 Hz) on GJIC suppression induced by 0.4mT 50Hz MF. The results showed that the noise MF with the same intensity of 50 Hz MF can block the suppression induced by 0.4mT 50 Hz MF. The results demonstrated that incoherent noise MF could inhibit the biological effects produced by coherent MF.