Effect of extremely low frequency electromagnetic fields on α-Lactalbumin and Sulindac treated colon cancer cells: ELF-EMF and colon cancer

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Abstract

Aim: In this research, we aimed to investigate the effects extremely low frequency electromagnetic fields (ELF-EMF) on proliferation and apoptosis during treatment of primary and metastatic colon cancer cell lines.

Material and Methods: Colon cell lines; COLO-320, COLO-741 and as control mouse fibroblast (STO) cells were cultured in 24 wells of tissue culture plate. Both COLO-320 and COLO-741 cells were treated with α-lactalbumin, sulindac and α-lactalbumin + sulindac. The cells from all groups were exposed to 60 Hz ELF-EMF for 48 hours. For cytotoxicity analyses, the cells were collected and analyzed with ELISA. The cells were fixed in 4% paraformaldehyde for 30 minutes. Cell proliferation was analyzed by evaluating of anti-Ki-67 distribution using indirect immunohistochemistry, cell death were evaluated using TUNEL assay.

Results: After TUNEL assay, the TUNEL positive cells were detected in all treated and control groups. However, the number of apoptotic cells was increased after treatment with α-lactalbumin and EMF exposure on COLO-320 cells. The apoptotic cells were less in STO cells. The distribution of Ki-67 was also detected in all groups, but, there were more Ki-67 immunoreactivity in COLO-741 cells than other groups. The cell cytotoxicity was also increased after EMF exposure in all groups.

Discussion: Our results suggested that the EMF exposure may increase the effects of α-lactalbumin on primary colon cancer cell lines. However, the proliferation of both cancer and control cells were not affected. The EMF exposure may trigger apoptotic pathway in primary colon cancer cell lines.

Keywords: Electromagnetic Fields, Apoptosis, Proliferation, Colon cancer, α-lactalbumin, sulindac, TUNEL assay

Introduction

Electric and magnetic fields (EMF) are produced when electric power is generated, transmitted, and used. Worries about even if EMF could negatively affect human health were raised at the beginning by epidemiologic studies reported in the late 1970s, and since the 1980s, data’s on EMF have been widely announced in the popular press. Electromagnetic fields (EMF) such as those from electric power transmission and distribution lines (50/60 Hz) have been associated with increased risk of childhood leukemia, cancer of the nervous system, lymphomas and other cancer types. At the same time humans are daily exposed to electromagnetic field (EMF) resources. In 2002, the International Agency for Research on Cancer (IARC, 2002) categorized extremely low frequency (ELF-EMF) (including the power frequencies of 50 and 60 Hz) magnetic fields as "possibly carcinogenic" [1]. Some parts of studies have marked possibly contrary effects induced by EMF [2]. On the other hand some researchers have showed beneficial effects of EMF in biological aspects [3].
Due to colon cancer is the second most frequent reason in the cancer-related deaths in the world, in this study; we have studied effects of electromagnetic fields during in vitro treatment of colon cancer cells. It is reported that colon cancer is the 4th most frequent cancer in males and the 5th most frequent cancer in females [4]. There are a lot of studies about colon cancer which are trying to find new therapeutic approach by various combinations with drugs or other treatment manners. We used two drugs for combination therapy which are Sulindac and alpha Lactalbumin. Researchers have showed that non-steroidal anti-inflammatory drugs and highly selective COX-2 inhibitors hold promise as anticancer agents. Giardiello et al showed that Sulindac effectively inhibits growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP) [5]. In the 2008, Researchers published that Sulindac may be more effective than Celecoxib which is Celecoxib, highly selective COX-2 inhibitor, when it is combined with DMFO in FAP patients who has colectomy operation [6]. On the other hand α-lactalbumin has been used for combination therapy. α-lactalbumin is the most generous protein in human milk and is renowned as a coenzyme in lactose synthesis. Researchers have found that when α-lactalbumin and oleic acid are used together as a complex which is called as HAMLET, they have effects on tumor cell viability. This molecular complex induces apoptosis in cancer cells but normal differentiated cells are resistant to HAMLET [7-9].

Living systems are under pressure of a lot of factor, which some of these factors compels to cells to cancer formation such as air, water pollutants and electromagnetic fields. Response to cancer therapy is also dependent to environmental factors. For this reason, in this study, effects of ELF-EMF on the α-lactalbumin and sulindac treatments were investigated.

### Material and methods

#### Cell Culture

Semi-adhesive, Human Primary colon cancer cell line Colo-320 (HTL95027, Interlab Cell Line Collection, Genova, Italy) and Adhesive, Metastatic human colon cancer cell line Colo-741 (HTL95008, Interlab Cell Line Collection, Genova, Italy) were incubated in %5 CO₂, 37 ºC, Humidified Atmosphere conditions.

![Figure 1. Semi-adhesive, Human Primary colon cancer cell line: Colo-320 (HTL95027, Interlab Cell Line Collection, Genova, Italy) . Adhesive, Metastatic human colon cancer cell line: Colo-741 (HTL95008, Interlab Cell Line Collection, Genova, Italy)](image1)

Extremely Low Frequency Electromagnetic Field ELF-EMF

For generating electromagnetic field, Helmholtz coils (Leibold Elektrotechnik, Germany) and signal generator (Philips) equipment was used. Diameter of Helmholtz coil measured as 20 cm and distance between two coils measured as 10 cm. Configuration of field is designed by using non-pulsed alternative current at 60 Hz and 15 volt.

Field that generated by method described above, was measured and characterized by

![Figure 2. Helmholtz Coils and Power supply system for ELF-EMF](image2)
using Gauss meter measuring equipment. According to measurements, electric field value was 10 v/m and magnetic field value was 15 mT in the middle of the coils where culture flask was placed during exposure time (Figure 2).

Results

ELISA Results
There are certain implications of cellular responses influenced by EMF in vitro.

A tremendous number of cellular components, cellular signals and cellular systems can probably be affected by EMF. However, EMFs are contrary to cause DNA damage directly because it has not been showed any data like that; most studies have been developed to investigate its effect on the cellular membrane, several gene expression, and signal transduction pathways. In our study, firstly we tested cytotoxicity of combination drug treatment and EMF effect on primary, metastatic colon cancer cells and mice STO fibroblast cells as a normal cell group by ELISA. We have clearly seen that EMF treated all groups have more cytotoxic effect than other groups (Figure 3). Cells were cultured in 24 wells of tissue culture plate. After sub-culturing of cells, they were cultured 24 hours. Both COLO-320 and COLO-741 cells were treated with α-lactalbumin, sulindac and combined treatment (α-lactalbumin + sulindac). For control, we used untreated COLO-320, COLO-741 and STO cells. The cells from all groups were exposed to 60 Hz power line EMF for 48 hours. Every 2 hours, EMF was exposure for 15 minutes, during 48 hours, 8 exposures were applied. The TUNEL positive cells (apoptotic cells) were detected, which were identified with brown nucleus, in all groups. However, there were more apoptotic cells in COLO-320 treated with α-lactalbumin and EMF exposure applied group when we compare with other groups (Figure 4). Apoptotic cells were less in STO cells (Figure 5, 6). There was some brown looking cells observed but they were death cells in the culture.

Ki-67 Immunoreactivity

Ki-67 is a cancer antigen that is found in growing and proliferating cells. The distribution of Ki-67 was also detected in all groups, but, there were more Ki-67 immunoreactivity in COLO-741 cell line which is characterized metastatic behavior (Figure 7).
Figure 5. TUNEL Assays of COLO cells (x200)

Figure 6. TUNEL Assay in STO Cells

Figure 7. Ki-67 Immunoreactivity in COLO-741 cell line
Discussion

Effects of electromagnetic field in normal and cancer cells are under investigation by researchers. But still there are a few studies about EMF effect combined with chemotherapeutics and other neo-adjuvant agents. Especially, in this study the behavior of primary or metastatic tumor cells may be different; therefore, the effective treatment can be important to cure for cancer. Our results suggest that combination treatment of EMF and α-lactalbumin is more effective when we compare with other groups. Svanborg and her colleagues have identified HAMLET as a new perioral agent for colon cancer prevention and treatment [10]. EMF can enhance α-lactalbumin induced apoptosis. Ki-67 immunoreactivity of COLO-741 cell line was more than other groups. This data can provide that metastatic colon cancers have high proliferative rate. This high degree of proliferation in metastatic colon cancer cells can minimize exposure of EMF. Although no change in colonic cell line proliferation, the balance between proliferation and apoptosis in the direction of increased apoptosis may be appropriate for treatment in in vitro condition; this effect may be analyzed also in vivo applications.

Conflict of Interest

The authors declared that they had no conflicts of interest.

References


