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Variable E-Cadherin Expression in a MNU-Induced Colon Tumor Model in Rats Which Exposed with 50 Hz Frequency Sinusoidal Magnetic Field

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TUNCEL, H., SHIMAMOTO, F., CAGATAY, P. and KALKAN, M.T. *Variable E-Cadherin Expression in a MNU-Induced Colon Tumor Model in Rats Which Exposed with 50 Hz Frequency Sinusoidal Magnetic Field.* Tohoku J. Exp. Med., 2002, **198** (4), 245-249 —— Inactivation of the E-cadherin system by multiple mechanisms, including both genetic and epigenetic events, plays a significant role in multistage carcinogenesis. We have investigated the effects of sinusoidal electromagnetic fields (SMF) on E-cadherin expression in an MNU (N-methyl-N-nitrosurea)-induced colon tumor model. Male wistar albino rats were used for the study. The rats were classified into four groups: I (MNU), II (SMF+MNU), III (SMF) and IV (control). After administered at MNU in 1st and 2nd groups, 2nd and 3rd groups were exposed to a sinusoidal magnetic field (SMF, 50 Hz, 5 mT) for 6 hours/day for 8 months. The expression of E-cadherin were examined in four groups of rat colon tissues by immunohistochemistry on paraffin sections. For immunohistochemical analysis, the labeled streptavidin biotin method was performed using a Vectastain Universal Quick Kit with microwave accentuation. Fisher's exact test was used for statistical analysis between proportions. Immunohistochemical studies of E-cadherin expression in this model demonstrated significant differences for cytoplasmic expression pattern. These results suggest that the electromagnetic fields result in significant alterations in cell adhesion mechanisms. This study has implications for understanding the role of fields in cell detachment in cancer metastasis. Further work is required to determine the

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relative effect of the magnetic fields on these phenomena. ———— E-cadherin; carcinogenesis; electromagnetic fields; immunohistochemistry
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Electric and magnetic fields associated with the production, transmission and use of electricity are ubiquitous in industrialized societies. These fields are predominantly of low frequency (60 Hz in the USA, 50 Hz in Europe and 50 or 60 Hz in Japan) and generally of low intensity. Electric fields exist when there is electric potential in a line, while magnetic fields exist only when there is current flow. Since both electric and magnetic fields often occur together and are interactive, these fields have often been referred to as electric and magnetic fields, or EMFs. Trees, walls and other objects shield electric fields while magnetic fields usually penetrate non-ferrous material. Thus, most residential exposure is to magnetic fields.

Recent research has focused on potential adverse health effects of exposure to magnetic fields (Anderson et al. 1999). Colon carcinogenesis is a representative multistep tumorigenesis that includes events of genetic alterations. Cell adhesion, particularly the cadherin-catenin system, has been a focus of considerable interest because of its potential role in the development of colon cancer.

E-cadherin has been demonstrated to induce growth suppression and decrease the invasiveness of cancer cells and thus has been proposed to be a tumor suppressor gene. The ability of E-cadherin to mediate cell-cell contact and contact inhibition presumably accounts for its antitumor effects, which are attributed to the extracellular domain of the protein (Sasaki et al. 2000).

E-cadherin is a transmembrane protein responsible for homophilic binding between epithelial cells and is necessary for establishing cellular polarity. Mutations in E-cadherin are frequent in metastatic disease and may be a rate-limiting step in the progression from adenoma to carcinoma. Chimeric transgenic mouse models that alter the expression of E-cadherin exhibit changes in proliferation, migration, and apoptosis (Joo et al. 2000; Sasaki et al. 2000).

Our target to investigate that the effects of sinusoidal electromagnetic fields (SMF) on E-cadherin expression by immunohistochemical methods. To address this aim, we performed an experimental colon tumor model in rats by N-methyl-N-nitrosurea (MNU).

Material and Methods

Animals

We used 30 male Wistar albino rats 2–2.5 months age that were obtained from DETAE (Institute for Experimental Medicine Research and Application, Istanbul, Turkey). A duration of one week before the experimental period was held for the compliance and controls of experimental animals. Animals were divided into 4 groups as shown in Table 1.

Rats were housed five per polycarbonate cage (cages standard for all groups were cleaned twice a week). Water and pelleted diet were available ad libitum. The animal room was checked for temperature, humidity and light status (12 : 12 light-dark cycle). Temperature was maintained between 23 and 25°C and relative humidity between 35 and 65%.

Table 1. *Experimental groups with animal numbers*

	Groups	Number of animals
1	MNU	10
2	SMF+MNU	10
3	SMF	5
4	Control	5

Carcinogen administration

60 mg MNU (N-methyl-N-nitrosurea) (Sigma Chemical Co., Dorset, UK) was dissolved in 6 ml sterile isotonic solution (0.9% NaCl). Prepared solutions were given i.r. (intra-rectal) to the MNU and SMF+MNU groups as 0.2 ml/per animal. In the procedure a number 8 feeding tube was inserted 6 cm into the rectum and solution was administered. The same procedure was applied to the SMF and Control groups but 0.2 ml/per animal sterile isotonic solution was administrated instead. This procedure was repeated once a week for 10 weeks.

Application of magnetic field

Twelve serially connected selenoid bobins made of copper which had 860 turning and a iron core. Bobins were connected to city electric. When current passed through the bobins the magnetic field in the cages was measured as 5 mT. For the measurement a Hall Effect teslameter of Leybold Heraous 54 050 model was used. After first administered at MNU in MNU and SMF+MNU groups, SMF+MNU and SMF groups were exposed to a sinusoidal magnetic field (SMF, 50 Hz, 5 mT) for 6 hours/day for 8 months.

Histological processing

The animals from all groups were killed 8 months after the first i.r. injections of MNU. Immediately after the sacrifice, colons were removed, cut open along its longitudinal axis, and fixed flat in 10% buffered formalin for 24 hours at room temperature. We photographed and/or checked the aberration of the surface of colon mucosa.

Immunohistochemistry and evaluation

For immunohistochemical analysis, the labeled streptavidin biotin method was performed using a Vectastain Universal Quick Kit (Vector Laboratories, Burlingame, CA, USA) with microwave accentuation. The paraffin-embedded sections were heated for 30 minutes at 65°C, deparaffinized in xylene, and rehydrated through graded alcohols at room temperature. A 0.05 M Tris-HCl buffer (pH 7.6) was used to prepare solutions and for washes between various steps. Incubations were performed in a humidified chamber. Four-μm-thick sections were treated for 40 minutes at room temperature with 2% BSA and incubated overnight at 4°C with primary antibodies against E-cadherin (diluted 1 : 1000; Transduction Laboratories, Lexington, KY, USA). For each case, negative controls were performed on serial sections. On the control section, incubation with the primary antibody was omitted. Horseradish peroxidase activity was visualized by treatment with H_2O_2 and diaminobenzidine for 5 minutes. At the last step, the sections were weakly counterstained with hematoxylin.

A semiquantitative analysis of the immunohistochemistry was performed to determine the approximate percentage of cells expressing E-Cadherin as follows: absent (−), 0% expression; slight (+), up to 20% of cells positive; moderate (++), 21% to 50% of cells positive; and strong (+++), more than 50% of cells positive.

Histopathological examination

Histological evaluation was performed by routine procedures with H & E staining. The stained sections were examined for grade of histological abnormality.

Statistical analysis

Fisher's exact test was used for statistical analysis between proportions.

RESULTS

The results of the E-cadherin immunoreactivity investigations are summarized in Tables 2 and 3. Immunohistochemical analysis of nuclear E-cadherin (ECAD-N) staining in different groups are shown in the Table 2.

TABLE 2. *Immunohistochemical analysis of nuclear E-cadherin (ECAD-N) staining in different groups*

Groups	(−) (%)	(+) (%)	(++) (%)	(+++) (%)
MNU	13 (72.2)	5 (27.8)	0	0
SMF+MNU	13 (56.5)	6 (26.1)	3 (13)	1 (4.3)
SMF	4 (57.1)	0	3 (42.9)	0
Control	5 (100)	0	0	0

$p=0.103$. *n*, nuclear staining.

TABLE 3. *Immunohistochemical analysis of cytoplasmic E-cadherin (ECAD-C) staining in different groups*

Groups	(−) (%)	(+) (%)	(++) (%)	(+++) (%)
MNU	3 (16.7)	15 (83.3)	0	0
SMF+MNU	1 (4.3)	16 (69.6)	5 (21.7)	1 (4.3)
SMF	2 (28.6)	4 (57.1)	1 (14.3)	0
Control	5 (100)	0	0	0

$p=0.0001$. C, cytoplasmic staining.

Nuclear E-cadherin expression level in MNU group was found lower than SMF+MNU and SMF groups. But differences are not statistically significant ($p=0.103$). As shown in the Table 3, we found that statistically significant ($p=0.0001$) differences for cytoplasmic expression pattern of E-cadherin. In contrast the nuclear pattern of E-cadherin, cytoplasmic E-cadherin (ECAD-C) staining in MNU group was found higher than SMF group. The results of the present study indicate that the electromagnetic fields result in significant alterations in cytoplasmic E-cadherin expression in our model.

DISCUSSION

Several epidemiological and laboratory studies have suggested that power-line frequency (50–60 Hz) magnetic fields (MF) may increase the risk of certain cancers, most likely by a tumor-promoting or co-promoting effect. Overall, the ultimate aim of the studies is to understand the mechanisms of this effect (or effects), and to find ways to prevent and cure the disease. Because cancers are regarded as diseases caused by the disruption of homeostasis, re-establishing homeostasis is a logical approach to reversing the malignancy.

It has long been known that cell-cell adhesiveness is generally reduced in human cancers. Tumor cells are dissociated throughout the entire tumor masses of diffuse-type cancers, whereas those of solid tumors with high metastatic potentials are often focally dissociated or dedifferentiated at the invading fronts. Thus, both irreversible and reversible mechanisms for inactivating the cell adhesion system appear to exist (Hirohashi et al. 1998; Heimann et al. 2000).

Cadherins play a crucial role in epithelial morphogenesis and mediate intercellular adhesion. These receptors bind catenins and are involved in signal transduction pathways that regulate cell growth and apoptosis, and are frequently down-regulated in invasive and metastatic carcinomas (Bindels et al. 2000).

Altered E-cadherin expression has been suggested to be of prognostic significance in breast cancers and to correlate with tumor subtype and grade. High-grade carcinomas

rarely revealed full expression and had a high incidence of aberrant nuclear localization of E-cadherin (Wang et al. 2000; Groosa et al. 2001; Sauer et al. 2001; Vizirianakis et al. 2002).

Immunohistochemical studies of E-cadherin expression in this model demonstrated significant differences for cytoplasmic expression pattern. Our results suggest that the electromagnetic fields result in significant alterations in cell adhesion mechanisms. This study has implications for understanding the role of fields in cell detachment in cancer metastasis. Further work is required to determine the relative effect of the magnetic fields on these phenomena.

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