P-selectin expression in a colon tumor model exposed by sinusoidal electromagnetic fields

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Abstract. P-selectin is mainly involved in the initial process of tumor cell adhesion to platelets. The aim of the present study was to determine the expression level of P-selectin in a colon tumor model affected by sinusoidal electromagnetic fields (SMF). Male Wistar albino rats aged 2-2.5 months were used. The animals were divided into the I [N-Methyl-N-Nitrosurea (MNU)], II (SMF-MNU), III (SMF) and IV (control) groups. The rats were housed five per polycarbonate cage. Sixty milligrams of MNU was dissolved in 6 ml sterile 0.9% NaCl. Prepared solutions were administered intra rectally (i.r.) to the 1st and 3rd groups as 0.2 ml/per animal. The same procedure was applied to the 2nd and 4th groups, although 0.2 ml/per animal sterile isotonic solution was administered instead. This procedure was repeated once a week for 10 weeks. Following the administration of MNU, the 2nd and 3rd groups were exposed to a sinusoidal magnetic field (SMF, 50 Hz, 5 mT) for 6 h/day for 8 months. P-selectin expression of the four groups of rat colon tissues was determined using immunohistochemistry on paraffin sections. The labeled streptavidin biotin method was performed. Fisher's exact test was used for differences between proportions. Results showed that there was no statistically significant ($P>0.05$) change in the expression level of P-selectin. However, this result should be verified by both *in vivo* and *in vitro* experiments to determine the effects of the magnetic fields on P-selectin.

Introduction

One of the major man-induced enviromental changes, which occurred in the last century, is the continued use of oscillating electromagnetic fields (EMF). These fields are generated for communication purposes, via the radio, television and cell phones, or as by-products of technology established for visualization purposes as in the case of computer and television screens, or from power lines. Electromagnetic radiation at this wavelength range is considered harmless and therefore insignificant as a pollutant when compared to the health dangers associated with, for instance, smog-generating fuel. However, with the identification of major sources of enviromental pollution investigation into the biological effects of these EMF is necessary (1).

Findings of previous studies have demonstrated variations in cell proliferation and apoptosis subsequent to exposure to ELF fields (2,3), while such changes have not been identified in other studies (4). Thus, a careful examination of the literature in this field suggests that more studies examining the role of ELF fields in cancer should be conducted. Cancer cell proliferation and apoptosis, invasion, and metastasis are complex phenomena controlled by an even more complex series of pathways, which communicate mutually through a number of signaling cascades. Thus, cell adhesion molecules (CAMs) play a key role in this process. CAMs and their receptors mediate cell-cell and cell-matrix interactions and are key in tumor growth, invasion, metastasis and death. The CAMs principally involved in these processes are those directed against important components of the extracellular matrix such as fibronectin, collagen, laminin, hyaluronan, heparan sulfate and elastin. Although a limited number of studies examining the effects of magnetic fields on cell adhesion are available, important insights into this issue have been previously provided (5).

Stimulated endothelial cells and activated platelets express P-selectin (CD62P), a member of the selectin family of CAMs, which interacts with P-selectin glycoprotein ligand 1 (PSGL-1, CD162) for leukocyte rolling on the stimulated endothelial cells as well as for the heterotypic aggregation of activated platelets onto leukocytes (6). Cross-linking of PSGL-1 by P-selectin also primes leukocytes in an intracellular manner for cytokine and chemoattractant-induced integrin activation for the firm adhesion of leukocytes. Furthermore, P-selectin mediates the heterotypic aggregation of activated platelets to cancer cells and adhesion of cancer cells to stimulated endothelial cells. Findings of *in vivo* experimental studies (7)

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indicate that P-selectin is important in the growth and metastasis of cancers. In this regard, the elucidation of the molecular mechanisms responsible for the regulation of the expression of these different P-selectin ligand molecules in various human cancer cells may yield noteworthy results (6,7).

The aim of the present study was to determine the expression level of P-selectin in a colon tumor model that was affected by sinusoidal electromagnetic fields (SMF).

Materials and methods

Animals. Healthy male Wistar albino rats, aged 2-2.5 months, were employed in the present study. A duration of one week prior to the experimental period was maintained for the compliance and controls of experimental animals. Animals were divided into four groups (Table I).

The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996).

Rats were housed five per polycarbonate cage (standard cages for all groups were cleaned twice a week). Water and pelleted diet were available *ad libitum*. The animal room was maintained between 23 and 25˚C and relative humidity was between 35 and 65%.

Carcinogen administration. Sixty milligrams N-Methyl-N-Nitrosurea (MNU) (Sigma Chemical Co., Dorset, England) was dissolved in 6 ml sterile isotonic solution (0.9% NaCl). Prepared solutions were administered intra rectally (i.r.) to the MNU and SMF+MNU groups as 0.2 ml/per animal. A number 8 feeding tube was inserted 6 cm into the rectum and the solution was administered. The same procedure was applied to the SMF and control groups, although 0.2 ml/per animal sterile isotonic solution was administered instead. This procedure was repeated once a week for 10 weeks.

Application of magnetic field. Twelve serially connected copper solenoid coils generated the magnetic fields, each having 560 turns. The cores of the coils were filled with soft iron rods and tightened to increase the magnetic flux intensity. Plastic cages were used to avoid the magnetic field strength distribution being affected. The coils were vertically placed and the cages were held 1.2 cm above the coils to hinder them from probable vibration. To protect the cages from heat of the coils, 1 cm thick wooden plates were placed between the coils and cages. The coils were connected to the 220 V 50 Hz sinusoidal city electric systems. When current passed through the coils, the magnetic flux intensities were measured at five different places inside the cages to be 5 mT. For measurement, we used a Leybold Heraeus 54050 model Hall effect teslameter.

Histological processing. Animals from all the groups were sacrificed 8 months after the first i.r. injections of MNU. Immediately after sacrifice, colons were removed, sectioned along the longitudinal axis, and fixed flat in 10% buffered formalin for 24 h 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 min at 65˚C, deparrafinized 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. Sections (4 μm) were treated for 20 min at room temperature with 5% BSA and incubated overnight at 4˚C with primary antibodies against P-selectin (BD Pharmingen, San Diego, CA, USA). For each case, negative controls were performed on serial sections. Incubation with the primary antibody was omitted for the control group. Horseradish peroxidase activity was visualized by treatment with H_2O_2 and diaminobenzidine for 5 min. The sections were then weakly counterstained with hematoxylin.

A semiquantative analysis of the immunohistochemistry was performed to determine the approximate percentage of cells expressing P-selectin as follows: absent (-), 0% expression; slight (+), up to 20% of cells positive; moderate (++), 21-50% of cells positive; and strong (+++), >50% of cells positive.

Histopathological examination. Histological evaluation was performed by routine procedures with hematoxylin and eosin (H&E) staining. The stained sections were examined for grade of histological abnormality.

Table I. Study groups with number of animals.

Group	No.
MNU	7
SMF+MNU	7
SMF	9
Control	5

MNU, N-Methyl-N-Nitrosurea; SMF, sinusoidal magnetic field.

Table II. Immunohistochemical analysis of P-selectin stainning in the study groups.

	Staining score frequency (%)			
Groups	-	+	++	+++
MNU	7 (38.9)	6 (33.3)	5 (27.8)	0 (0.0)
SMF+MNU	12 (52.2)	5 (21.7)	5 (21.7)	1 (4.3)
SMF	5 (71.4)	0 (0.0)	2 (28.6)	0 (0.0)
Control	5 (100)	0 (0.0)	0 (0.0)	0 (0.0)

P>0.05 for all comparisons between groups. MNU, N-methyl-N-nitrosurea; SMF, sinusoidal magnetic field.

Results

Healthy, male, Wistar albino rats, aged 2-2.5 months, were were divided into four groups and the colons were examined.

No appreciable change in food consumption was observed among the different groups of rats. Fisher's exact test was used to calculate the differences between proportions. No statistically significant (P>0.05) changes in the expression level of P-selectin were identified (Table II).

Discussion

Electric and magnetic fields associated with the production, transmission and use of electricity are ubiquitous in industrialized society. These electric and magnetic fields are predominantly of low frequency (60 Hz in the US, 50 Hz in Europe and 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 (8). 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.

Previous studies have focused on the potential adverse biological effects of exposure to magnetic fields. The residential exposures in most homes to magnetic field intensities are <2 milligauss (mG), which is equivalent to 0.2 microtesla although some areas in homes may exceed this field intensity. In certain industrial settings, the mean workplace magnetic field exposure may exceed 10 mG (9).

Recent studies have investigated the effects of EMFs concurrently with technological advances. While several studies suggested that EMF exposure induces immune cell activation and DNA damage (10,11), the findings of other studies indicated that the effects of EMF inhibit preneoplastic lesions, resulting in the reduction of cell proliferation (3). A number of studies focused on the potential effects of EMF at cancer treatment (12,13).

Metastasis is a cascade of cell events by which cancer cells establish new colonies at distant sites in the body (14,15). It comprises multiple, consecutive steps. Several CAMs are involved in the various stages of cancer metastasis (16). P-selectin has been shown to bind to several human cancers and human cancer-derived cell lines, such as colon cancer, lung cancer including small-cell lung cancer, breast cancer, malignant melanoma, gastric cancer, neuroblastoma and adenoid cystic carcinoma of the salivary gland. *In vivo* experimental evidence has indicated that P-selectin is important in the growth and metastasis of cancers (7,17,18). Of note therefore is the elucidation of the molecular mechanisms responsible for the regulation of the expression of these different P-selectin ligand molecules in various human cancer cells (6,19,20).

The expression of P-selectin is regulated at the transcriptional level, with tumor necrosis factor-α inducing P-selectin expression in mouse and bovine endothelial cells (21,22). By contrast, interleukin-4 and ontostatin M induce P-selectin expression in human umbilical vein endothelial cells, which can last up to 72 h (23). However, the mechanisms involved in this pathway remain to be determined (6,7).

Immunohistochemical studies of P-selectin expression in this model demonstrated no statistically significant (P>0.05) differences between the groups. Zhang *et al* (24) have shown no effects of pulsed EMF on the expression of another CAM, integrin, in osteosarcoma cell lines. Further studies should be performed to verify the findings of this study, including new methods for P-selectin detection *in vivo* and *in vitro*. Additionally, the relative effect of the magnetic fields on this molecule and the correlation with metastatic events should be detemined.

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References

1. Giacomoni PU and Rein G: Factors of skin ageing share common mechanisms. Biogerontology 2: 219-229, 2001.
2. Tofani S, Barone D, Cintorino M, *et al*: Static and ELF magnetic fields induce tumor growth inhibition and apoptosis. Bioelectromagnetics 22: 419-428, 2001.
3. Jiménez-García MN, Arellanes-Robledo J, Aparicio-Bautista DI, Rodríguez-Segura MA, Villa-Treviño S and Godina-Nava JJ: Anti-proliferative effect of extremely low frequency electromagnetic field on preneoplastic lesions formation in the rat liver. BMC Cancer 10: 159, 2010.
4. Merola P, Marino C, Lovisolo GA, Pinto R, Laconi C and Negroni A: Proliferation and apoptosis in a neuroblastoma cell line exposed to 900 MHz modulated radiofrequency field. Bioelectromagnetics 27: 164-171, 2006.
5. Santini MT, Rainaldi G, Ferrante A, Indovina PL, Vecchia P and Donelli G: Effects of a 50 Hz sinusoidal magnetic field on cell adhesion molecule expression in two human osteosarcoma cell lines (MG-63 and Saos-2). Bioelectromagnetics 24: 327-338, 2003.
6. Chen M and Geng JG: P-selectin mediated adhesion of leukocytes, platelets, and cancer cells in inflammation, trombosis, and cancer growth and metastasis. Arch Immunol Ther Exp 54: 75-84, 2006.
7. Läubli H and Borsig L: Selectins promote tumor metastasis. Semin Cancer Biol 20: 169-177, 2010.
8. Miller FJ and Schroeer D: College Physics. 6th edition. Harcourt Brace Jananovich, San Diego, 1987.
9. Theriault G, Goldberg M, Miller AB, *et al*: Cancer risks associated with occupational exposure to magnetic fieldsamong electric utility workers in Ontario and Quebec, Canada, and France: 1970-1989. Am J Epidemiol 139: 550-572, 1994.
10. Simko M and Mattsson M-O: Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. J Cell Biochem 93: 83-92, 2004.
11. Huang L, Dong L, Chen Y, Qi H and Xiao D: Effects of sinusoidal 50 Hz magnetic field on viability, cell cycle and apoptosis of HL-60 cells. Eur Phys J Appl Phys 35: 217-221, 2006.
12. Berg H, Günther B, Hilger I, Radeva M, Traitcheva N and Wollweber L: Bioelectromagnetic field effects on cancer cells and mice tumors. Electromagn Biol Med 29: 132-143, 2010.
13. Barbault A, Costa FP, Bottger B, Munden RF, Bomholt F, Kuster N and Pasche B: Amplitude-modulated electromagnetic fields for the treatment of cancer: discovery of tumor-specific frequencies and assessment of a novel therapeutic approach. J Exp Clin Cancer Res 28: 51, 2009.
14. Albelda SM and Buck CA: Integrins and other cell adhesion molecules. FASEB J 4: 2868-2880, 1990.
15. Miyasaka M: Cancer metastasis and adhesion molecules. Clin Orthop Relat Res 312: 10-18, 1995.
16. Huang YW, Baluna R and Vitetta ES: Adhesion molecules as targets for cancer therapy. Histol Histopathol 12: 467-477, 1997.
17. Gong L, Cai Y, Zhou X and Yang H: Activated platelets interact with lung cancer cells through P-selectin glycoprotein ligand-1. Pathol Oncol Res 18: 989-996, 2012.
18. Gong L, Mi HJ, Zhu H, Zhou X and Yang H: P-selectin-mediated platelet activation promotes adhesion of non-small cell lung carcinoma cells on vascular endothelial cells under flow. Mol Med Rep 5: 935-942, 2012.

19. Aruffo A, Dietsch MT, Wan H, Hellstrom KE and Hellstrom I: Granule membrane protein 140 (GMP 140) binds to carcinomas and carcinoma-derived cell lines. Proc Natl Acad Sci USA 89: 2292-2296, 1992.
20. Mannori G, Crottet P, Cecconi O, *et al*: Differential colon cancer cell adhesion to E-, P- and L-selectin: role of mucin type glycoproteins. Cancer Res 55: 4425-4431, 1995.
21. Hahne M, Jager U, Isenmann S, Hallmann R and Vestweber D: Five TNF-inducible cell adhesion mechanism on the surface of mouse endothelioma cells mediate the binding of leukocytes. J Cell Biol 121: 655-664, 1993.
22. Sanders WE, Wilson RW, Ballantyne CM and Beaudet AL: Molecular cloning and analysis of in vivo expression of murine P-selectin. Blood 80: 795-800, 1992.
23. Yao L, Pan J, Setiadi H, Patel KD and McEver RP: Interleukin 4 or oncostatin M induces a prolonged increase in P-selectin mRNA and protein in human cells. J Exp Med 184: 81-92, 1996.
24. Zhang D, Pan X, Ohno S, Osuga T, Sawada S and Sato K: No effects of pulsed electromagnetic fields on expression of cell adhesion molecules (integrin, CD44) and matrix metalloproteinase-2/9 in osteosarcoma cell lines. Bioelectromagnetics 32: 463-473, 2011.