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Evaluation of mutagenicity and cytotoxicity of intermediate frequency magnetic field using DNA repair deficient mammalian cells

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There are still a few studies on biological effects of intermediate frequency magnetic fields (IF-MF) which are generated by several devices such as inverter on electric train, IH cooker. In this study, the mutagenicity and the effect on cell growth of IF-MF (21 kHz) were evaluated. Continuous IF-MF exposure up to 3.9 mT was carried out by a newly developed IF-MF exposure system. As the results, there was no significant difference in mutation frequency in *HPRT* gene between sham and IF-MF exposure (2mT; 24hr) in CHO-K1. Furthermore, no significant difference in growth rate in CHO-K1 and its DNA repair deficient derivatives, xrs5 (*Ku86*), irs1SF (*XRCC3*) and EM-9 (*XRCC1*) was observed between sham and IF-MF exposure during growth period (up to 72hr). These results indicated that IF-MF did not affect mutation frequency and cell growth under the conditions in this study.

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中間周波磁界の変異原性およびDNA修復系欠損細胞を用いた毒性の評価

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鉄道やIH調理器などから発生する中間周波磁界 (IF-MF) の生体影響については，未だ知見が少ない。特に長期曝露に関する知見が少ないことから，我々は，数日間の曝露が可能な*in vitro*試験用のIF-MF曝露装置を開発してきた。本研究では，この曝露装置を用いて，IF-MFの変異原性および細胞増殖への影響を評価した。21kHz, 2mTのIF-MFにCHO-K1細胞を24時間曝露し，*HPRT*遺伝子の突然変異頻度を調べたところ，シャム曝露系とIF-MF曝露系では統計的に有意な差は観察されなかった。また，CHO-K1 細胞およびそのDNA 修復系欠損株 xrs5 (*Ku86*), irs1SF (*XRCC3*), EM-9 (*XRCC1*) にIF-MFを72時間曝露し，WST-1を用いて細胞増殖への影響を評価した。この結果，DNA修復系欠損の有無に関わらず，シャム曝露系とIF-MF曝露系では統計的に有意な差は見られなかった。このことから，本研究で曝露したIF-MF条件では，突然変異頻度および細胞増殖に影響を及ぼさないことがわかった。

本研究の一部は厚生労働科学研究費補助金，健康安全・危機管理対策総合研究事業によって行われた。

P-060

Development of colon tumors by benzo[*a*]pyrene in a mouse colitis model using dextran sulfate sodium

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We previously reported that the potency of mutagenesis does not necessarily correlate with carcinogenicity because the colon, a non-target organ for carcinogenesis, showed the highest mutation following treatment of a transgenic mouse (MutaMouse) with benzo[*a*]pyrene (BP). In the present study, we applied BP to a colon-colitis mouse model using dextran sulfate sodium (DSS) to examine whether or not colon tumors developed.

$CD2F_1$ male mice were administered BP at 125 mg/kg/day for 5 days, and then given 4% DSS twice in drinking water for 1 week over an interval of 2 weeks. These mice were sacrificed for pathological examination of the colon 14 and 17 weeks after the first administration.

Following treatment with BP and DSS, a large number of tumors were found in all the mice from 14 weeks onward after the first administration. In contrast, a limited number of tumors were observed in mice exposed to DSS, and neither a proliferative lesion nor a tumor was observed in mice exposed to BP alone, with the vehicle.

These findings suggest that gene mutations in stem cells or progenitor cells of the colon crypt as well as other factors, including inflammation, are involved in colon carcinogenesis.

Dextran sulfate sodiumマウス大腸炎モデルにおけるBenzo[*a*]pyreneによる大腸腫瘍の発生

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我々は既に，トランスジェニックマウス (MutaMouse) にBenzo[*a*]pyrene (BP) を投与し，発がんの非標的臓器である大腸に最も高頻度に変異が起きていたことから，BPの発がん性と変異頻度は必ずしも相関しない事を見出した。今回，Dextran sulfate sodium (DSS) による大腸炎モデルを用いて，大腸に腫瘍が発生するか否かを検討した。

BPを125 mg/kg/dayで5日間$CD2F_1$雄マウスに経口投与した後、2週間の休薬期間をはさんで4%DSSの1週間飲水投与を2回繰り返した。投与開始から14週後及び17週後に剖検し、大腸を病理学的に観察した。

BPとDSS処理により、全例のマウスに多発性の腫瘍が14週以降で観察された。一方、DSS単独群ではごく一部に少数の腫瘍が発生し、BP単独群及び媒体群には増殖性病変及び腫瘍はみられなかった。

以上より，大腸腫瘍の発生過程において，クリプト中の幹細胞あるいは前駆細胞における遺伝子変異のみならず、炎症を含めた他の要因が関与していることが示唆された。

In vitro 小核試験による中間周波磁界の生物影響評価

Evaluation of Biological effects of intermediate frequency magnetic fields using *in vitro* micronucleus assay

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【はじめに】近年、IH 調理器の普及が進み、家庭内で中間周波磁界（中間周波帯：300Hz～10MHz：WHO の定義による）が発生する環境が増加しているほか、電気鉄道においても、駆動用の主インバーター由来の中間周波磁界や極低周波磁界が低いレベルで環境中に発生していることから、中間周波の健康リスクに対する関心が高まっている。しかし、これまでに十分な実験データが蓄積されていないため、基礎的な知見を得ることが重要な課題となっている。本研究では、CHL/IU 細胞を用いた *in vitro* 小核試験による中間周波磁界の変異原性評価結果を報告する。
【方 法】中間周波磁界の曝露には、我々が開発した曝露装置を用いた。この曝露装置は、Meritt 型の電磁コイルおよび電磁コイル内部に設置した樹脂製の炭酸ガス培養器から成り、15 cm × 15cm × 15cm の空間に最大 3.9mT の磁界を 37±1℃以内の温度制御下で発生させ、生物試料を曝露することが可能である。
前培養した CHL/IU 細胞を 4×10^4cells/ml の細胞濃度で 90mm シャーレに 5ml ずつ分注し、4 群（磁界曝露群、疑似曝露群、陽性対照群（MMC0.04 μg/ml 処理）、インキュベータ対照群）に分けた。それぞれの条件で 24 時間培養した後、細胞を回収・固定した。その後、細胞懸濁液をプレパラートに滴下・乾燥し塗抹標本を作製したのち、ギムザ染色をおこない、標本あたり約 1,000 個の細胞を観察し、小核を保持する細胞の割合を調べた。細胞の増殖については、72 時間までの経時的な増殖を WST-1 法により測定した。すなわち、前培養した CHL/IU 細胞を 1×10^4cells/mlの濃度で 3 枚の 96well プレートに 100μl ずつ播種し、磁界曝露群、疑似曝露群、インキュベータ対照群に分けた。その後、24 時間ごとに各プレートの 6 個の well に 10μl の WST-1 溶液を分注し、2 時間各条件で培養した後、450nm の吸光度を用いて得られる生細胞の濃度により増殖量を測定した。磁界曝露条件として、周波数 21kHz、磁束密度 2.0、3.0 および 3.9mT を検討した。
【結果および考察】小核試験においては、各磁束密度における実験を最低 4 回以上行い、対照群と磁界曝露群の小核出現頻度を比較した。しかし、いずれの曝露条件であっても、磁界曝露群と対照群との間で統計学的に有意な差は見られなかった。また、増殖速度についても同様に、すべての曝露条件で磁界曝露の影響は認められなかった。これらのことから、本研究で検討した 21kHz, 最大 3.9mT（国際非電離放射線防護委員会（ICNIRP）の一般環境におけるガイドライン参考レベルの百倍以上）の中間周波磁界への長時間曝露は、CHL/IU 細胞において小核を誘導せず、また増殖阻害棟の影響もないことが明らかとなった。従って，通常 ICNIRP ガイドラインを超えることのない電気鉄道や IH 調理器から発生する環境中の中間周波磁界の遺伝毒性は無いか、あるとしても極めて小さい影響しか持たないと評価される。
【謝辞】本研究の一部は厚生労働科学研究費補助金、健康安全・危機管理対策総合研究事業によって行われた。

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2-1-07

乗用型農業機械から曝露される全身振動の検討

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【はじめに】農業従事者の腰痛有訴率は高く、その要因の一つとして乗用型農業機械から曝露される全身振動が挙げられる。その低減のためには曝露実態に基づいた対策が必要である。そこで、全身振動曝露の多様な条件下（車種や走行面等）での実態、および曝露低減のための課題を明らかにすることを目的として、農業機械からの振動を測定した。

【対象と方法】測定車両は、ある稲作専業農家が所有する3台のトラクター（33ps，46ps，50psで種々の作業機装着）、田植え機、コンバイン（全幅6条刈り）の農機5台（同一製造社製）と運搬用トラック1台とした。各々およそ半日間測定した。振動は座面上と座席取り付け基部に設置した直交3方向の振動加速度検出器で測定し、振動計を用いて1秒ごとのISO 2631-1に則った3方向周波数加重振動加速度実効値a_{wi}（i：鉛直z, 前後x, 左右y）を得、その合成値a_{sum}を算出した。鉛直方向の基部に対する座面上振動値比*SEAT*を求めた。座面振動検出器上に設置した着座スイッチと記録計を用いて着座と離席の時刻を把握した。車両動態測定にはハンディGPSを用いた。作業日誌より作業時間を把握した。a_{sum}と1日の作業時間より推定できた車両／走行路別の曝露時間を用いて8時間等価振動値A（8）を算出した。測定はいずれも2010年に滋賀県湖南地域で実施した。

【結果と考察】各作業機を装着した車両別／走行路別の振動値、*SEAT*、速度を表に示した。圃場でのa_{sum}（m/s²）は0.65～1.16、圃場以外では0.85～1.33であり、全身振動値が大きくて問題と考えられているフォークリフト（0.6～1.0）に比べても高値であった。圃場でのa_{wz}（m/s²）は0.29～0.60であった。圃場以外では0.52～1.10であり、圃場より高値であった。軟らかい圃場地面と低い走行速度がその理由であろう。圃場では水平方向振動が比較的大きく、すべて優越軸はなかった。稲の刈り取り脱穀を行った日（8時間作業）について、圃場／舗装路／その他条件での農機運転とトラック運転に分類し算出したA（8）は0.70 m/s²（振動曝露4時間50秒）であり、EU指令の要対策A（8）値0.50 m/s²を上回った。さらに、もみの袋詰め等の重筋作業も行っていたため、収穫作業期での腰部負担は大きいと考えられた。

表　車両／走行路別の全身振動値(m/s²)、*SEAT*および速度(km/h)

車種と作業	測定日	走行	a_{wz}	1.4 a_{wx}	1.4 a_{wy}	優越軸	a_{sum}	*SEAT*	速度
トラクター 46ps 畦塗り	4/15	舗装路	0.69	0.57	0.39	Non	0.98	0.63	-
		非舗装路	0.80	0.63	0.46	Non	1.11	0.61	-
		圃場	0.31	0.75	0.54	Non	0.98	0.77	-
トラクター 33ps 畝立て	4/26	舗装路	0.52	0.43	0.52	Non	0.85	-	10.1
		非舗装路	0.81	0.62	0.85	Non	1.33	-	8.9
		圃場	0.57	0.79	0.60	Non	1.14	-	2.6
トラクター 50ps 荒耕	5/11	舗装路	0.94	0.50	0.57	Z	1.21	1.15	16.1
		圃場	0.32	0.43	0.37	Non	0.65	0.78	2.0
田植え機 田植え	5/13	舗装路	1.10	0.53	0.51	Z	1.32	0.84	9.1
		圃場	0.29	0.65	0.65	Non	0.97	0.86	2.5
コンバイン 刈り取り＆脱穀	9/15	舗装路	0.99	0.56	0.56	Z	1.27	0.55	5.8
		圃場	0.59	0.71	0.68	Non	1.15	0.69	4.1
トラクター 46ps 通気透水穴形成	10/26	舗装路	0.82	0.74	0.70	Non	1.30	0.40	5.2
		圃場	0.60	0.83	0.53	Non	1.16	0.55	2.9
トラクター 46ps 耕起＆表播種	11/08	舗装路	0.77	0.48	0.58	Non	1.08	0.57	4.4
		圃場	0.44	0.49	0.44	Non	0.79	0.66	2.7
トラクター 33ps 圃場内周掘り	11/09	舗装路	0.75	0.35	0.41	Z	0.92	0.83	8.5
		圃場	0.39	0.45	0.42	Non	0.73	0.93	1.8
トラック 農機運搬	4/15	舗装路	0.31	0.26	0.32	Non	0.52	-	-

a_{wi}：周波数加重加速度実効値，a_{sum}：3方向 a_{wi} の合成値

2-1-08

哺乳類細胞を用いた中間周波磁界の変異原性評価

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【目的】日常環境において種々の電磁界に曝露する機会が増えたことにより，電磁界の健康影響に対する公衆の関心は高い。電気鉄道も動力源として電気を使用することから，電磁界の発生源の1つといえる。鉄道で発生する磁界は，静磁界から中間周波磁界（300Hz～10MHz，intermediate frequency magnetic field: 以下IF-MFとする）であるが，この中で，日常環境において特に曝露機会の多い極低周波数帯（商用周波数帯）の電磁界については，生体影響に関する研究が多く行われ，世界保健機関（WHO）の国際EMFプロジェクトをはじめとして健康リスク評価が進められている。一方，中間周波磁界はIH調理器などからも発生することが知られているが，その生体影響に関する研究は少なく，科学的知見の収集とそれに基づく健康リスク評価を行うことが課題となっている。

本研究では，IF-MFの生体影響に関する研究のうち，特に強磁界への長時間曝露に関する研究が少ないことから，哺乳類培養細胞を用いて細胞増殖および遺伝子突然変異への長時間曝露の影響を評価した。

【方法】本研究では，細胞に磁界を曝露するため，我々が開発したIF-MF曝露装置を用いた。この曝露装置は，周波数21kHzで最大3.9mTまでの磁束密度の磁界を発生することが可能である。また，コイルの中に炭酸ガスインキュベータを設置することにより，細胞の生育に適した温度および炭酸ガス濃度を維持可能となっている。供試細胞として，CHO-K1株およびそのDNA修復欠損株であるxrs5（*Ku86*），irs1SF（*XRCC3*），EM-9（*XRCC1*）を用いた。細胞の増殖過程で72時間，IF-MFを曝露し，24時間ごとに生存細胞数をWST-1法により定量し，細胞増殖に与える影響を調べた。また，CHO-K1株およびEM-9株を用いて，IF-MF曝露下での*HPRT*遺伝子の突然変異出現頻度を調べた。

【結果および考察】磁束密度2ならびに3.9mTのIF-MFの細胞増殖に対する影響を調べたところ，CHO-K1株およびDNA修復欠損株のどちらについてもIF-MF曝露群および非曝露群との間に統計的に有意な差は見られなかった。また，突然変異試験では，磁束密度2，3，3.9mTのIF-MFをCHO-K1株およびEM-9株に曝露した結果，IF-MF曝露による*HPRT*遺伝子の突然変異出現頻度への統計的に有意な影響は認められなかった。一方，陽性コントロールとして行った発がん性物質であるメタンスルホン酸メチル（MMS）処理群では，有意な突然変異出現頻度の上昇が観察された。以上の結果より，21kHzのIF-MFは，磁束密度3.9mT（国際非電離放射線防護委員会（ICNIRP）の一般環境におけるガイドライン参考レベルの百倍以上）を長時間曝露しても，本研究で用いたDNA修復を欠損する種々の細胞株に対して細胞増殖および遺伝子突然変異出現頻度への影響を及ぼさないことがわかった。従って，通常ICNIRPガイドラインを超えることのない電気鉄道やIH調理器から発生する環境中のIF-MFの変異原性はないか，あるとしても極めて小さいと考えられる。

【謝辞】本研究の一部は厚生労働科学研究費補助金，健康安全・危機管理対策総合研究事業によって行われました。

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Primary Challenge :

For an even safer and more secure railway *(Adverse health effects of electric and magnetic fields (EMFs) are still concerned in society. Railway systems are no exception. Our study aims to provide evidences by experimental results to explain safety of EMFs in railway environment to reduce anxiety among public. Therefore, we think that our study related to the challenge for an even safer railway.)*

Second Challenge :

Topic : Electromagnetic compatibility

Keywords : Health risk of electric and magnetic fields, human dosimetry, biological experiments

EMFS IN RAILWAY SYSTEM -EVALUATION OF BIOLOGICAL EFFECTS AND CURRENT TRENDS OF ITS HEALTH RISK ASSESSMENT-

Last three decades, possible health effects of electric and magnetic fields (EMFs) have been discussed. As an outcome, the World Health Organization (WHO) recently published a health risk assessment of EMFs below 100 kHz (Environmental Health Criteria 238). In this document, they recommend to implement adequate guidelines such as ICNIRP and IEEE for prevention of acute effects. To deal with this, an IEC standard for EMFs measurement in railway is under discussion. On the other hand, WHO also recommends further research in several themes to elucidate scientific uncertainty about chronic effects that is suggested with only weak evidences to date. However, there are few reports on biological effects of combined EMFs that exist in railway systems. Therefore, we are investigating mutagenic effects of combined MFs as the series of evaluation of biological effects of EMFs because mutation is a key role for carcinogenicity that is the most concerned effect by chronic exposure in public. In this study, mutagenicity was evaluated in mouse lymphoma assay (MLA) that is a part of the standard test battery for Genotoxicity in OECD. Mouse lymphoma derived L5178Y tk+/- 3.7.2C cells were exposed to combined MFs (up to static 5T and 50Hz, 1mT) for 48hr. Then the cells were incubated two weeks for assay of survival rate and mutation frequency in thymidine kinase (tk) gene (tk+/- to tk-/-). As the result, the survival rate that is one of representative indices of acute toxicity was not affected by exposure to all MFs

conditions. In addition, no significant difference was observed in the mutation frequency in tk gene between MF exposed and unexposed cells in all experimental conditions. These results suggest that exposure to combined MFs did not induced gene mutations, larger scale chromosomal changes, aneuploidy and others that could be detected by MLA. Based on this and our previous results, we've started experiments of combined magnetic field with three components (static, 50 Hz and 2 kHz as a realistic MFs condition in rolling stock) as further research. In conclusion with taking into account our results and practical situation that will be much weaker MFs than experimental conditions exist where person can be in railway environment, it would suggest that complicated MFs in railway have no or extremely small mutagenic potential that is not able to be detected in general assays for carcinogenicity, thus, it should not be considered as adverse health risk.

What's new

There is few biological studies about health risk of electric and magnetic fields (EMFs) generated in railway field. In response, we've already published several reports about it. In this conference, we will provide first evaluation data of mutagenicity in mammalian cells of complicated MFs that are typically generated in rolling stock. In addition, numerical evaluation of dosimetry in human whole body model, current trends of guidelines (revised ICNIRP guideline, etc), health risk assessment (WHO) and measurement standard (IEC) will be also presented.

Biography

Dr. Ikehata works for RTRI since 1990. His main research is to evaluate biological effects of magnetic fields for health risk assessment. He received His Ph.D. degree in biotechnology from Tokyo University of Agriculture and Technology in 2001. He was awarded the Young Scientist Award of the International Radio Science Union (URSI) in 2002. He is a council member of Japanese Occupational Health Society, a member of Japanese national committee of URSI-K and a member of International Committee on Electromagnetic Safety in IEEE. He has frequently attended WCRR since 1999.

Enclosed File :

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MODIFY MY ABSTRACT CONFIRM & SUBMIT

Biological effects of intermediate frequency magnetic fields -Development of exposure system and evaluate genotoxicity in vitro-

Masateru Ikehata[1], Sachiko Yoshie[2], Keiji Wada[3], Yukihisa Suzuki[4], Taiji Sakai[5], Kanako Wake[6], Satoshi Nakasono[7], Masao Taki[8] and Chiyoji Ohkubo[9]

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Abstract

To evaluate biological effects of intermediate frequency magnetic fields (IF-MFs), we developed an exposure system for *in vitro* study. This system is able to generate up to 3.9mT, 21kHz IF-MF under controlled culture condition. Toxicity and effect on frequency of micronucleus in CHL/IU cells by exposure to IF-MFs were investigated. As the results, we have not observed any significant effects by exposure to 21 kHz, up to 3.9 mT IF-MF in both experiment. This suggests that IF-MF is unlikely to cause adverse biological effects..

1. Introduction

Health effects of magnetic fields and electromagnetic fields (EMF) are still concerning in the society. Therefore, WHO is conducting the International EMF Project to evaluate health risk by exposure to EMF. WHO already published two environmental health criteria monographs for static fields (No. 232, in 2006) [1] and time-varying (below 100 kHz) extremely low frequency fields (No. 238, in 2007) [2].

On the other hand, various IF-MFs are generated in various situations such as cooking with IH hob, taking electric train, using RF-ID tags or IC cards. Especially, several kHz to 100kHz, However, health risk of IF-MF is inconclusive because there are few researches to date, thus further research in frequencies between 300 Hz to 100 kHz are recommended by WHO. In order to deal with this situation, we have launched new research project to estimate biological effects and health risks of exposure to IF-MF. In this study, we developed newly designed IF-MFs exposure system for *in vitro* test systems and started to evaluate biological effects.

2. Materials and Methods

Recently, it is becoming popular to use IH cooking apparatus in Japan. A time-varying magnetic field with frequency range 20 kHz to 100 kHz is used as the fundamental heating frequency and among those, a 20 kHz IF-MF is mainly used. Besides, industrial inverters (e.g. inverter used for VVVF electric train) are also used in these frequencies. From these devices, IF-MFs are possibly strayed in environment. Thus, around 20 kHz is primary target frequency in this study.

Existing guideline by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) that published in 2010 provided a reference level of exposure (27 μT in these frequencies range for public) [3]. Thus, we decided 3mT (about 100 times higher than reference level of ICNIRP guideline) was set as maximum field strength of IF-MFs. In order to achieve this strength and sufficient exposure space, Merritt coil was chosen as the coil configuration. Temperature, humidity and concentration of CO_2 was controlled using an incubator that made by resin that was located inside of the coil. We developed two identical units of the coil and also the incubator. We arbitrarily combined each unit and used one for exposure and the other for sham-exposed control.

For biological experiments, CHL/IU was used for micronucleus assay. To investigate the growth rates, 100 μl aliquot (10^4 cells/ml) of these cell lines poured into each well of 96-well plates and incubated for 24 hr prior to the exposure period, then the plates were exposed to IF-MF. After 0, 24, 48 and 72 hr, 10 μl of WST-1 premix

solution (TaKaRa Bio Inc., Japan) was added to 16 well each and then put back to the device, respectively. Absorbance at 450 and 630 nm was measured using microplate reader (Bio-Rad laboratories, Inc., USA) after incubation for 3 hr.

In *in vitro* micronucleus assay, CHL/IU cells are incubated in exposure apparatus with or without MFs for 24h in 90mm Petri dishes. After the exposure, cells are fixed and stained for counting micronucleus. The ratios of micronucleus formation are estimated by counting micronucleus in approximately 1,000 cells.

3. Results and Discussion

Newly developed exposure system is capable of generating 21 kHz, up to 3.9mT IF-MF (624 times higher than reference level (6.25μT) for public in existing ICNIRP guideline) within exposure space (150mmX150mmX150mm) within +/- 5% deviation.

Six 90mm Petri dishes or six 96 well test plates are able to be putted in the resin CO_2 incubator that located inside the Merrit coil. This water-circulated incubator was able to maintain temperature in exposure space at 37 +/- 1 °C with IF-MF exposure up to 3.9mT or without IF-MF. It was confirmed that the culture condition of this resin incubator was appropriate since CHL/IU cells revealed normal growth in the incubator without IF-MF. Thus, strong IF-MF exposure for cell culture was established for *in vitro* experiments, such as cell culture system using this exposure system (Fig. 1).

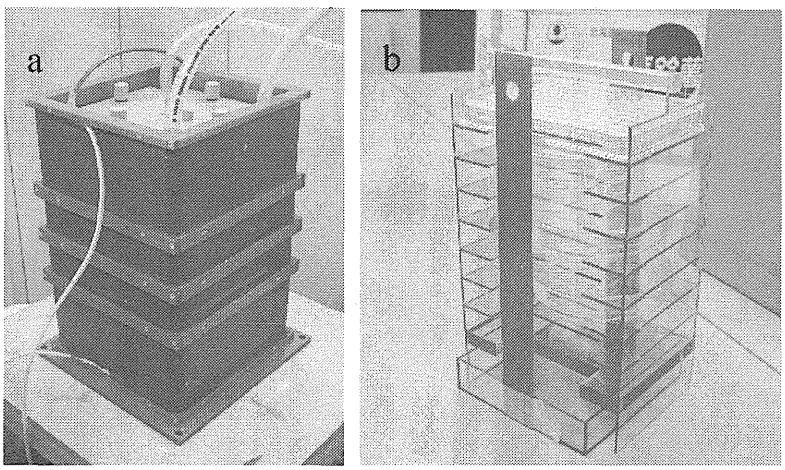

Fig. 1 Photographs of the exposure system. (a) : the Merritt coil and the incubator, (b): the rack used for the incubator.

In the biological experiments, growth rate under IF-MF exposure (up to 3.9mT) was examined in CHL/IU. We observed no significant effect on the growth rate up to 72 hr in all of the cells and there is no difference between exposed and sham exposed group. This result suggests IF-MF exposure does not affect cell growth. Result of CHL/IU cells were shown in Fig. 2.

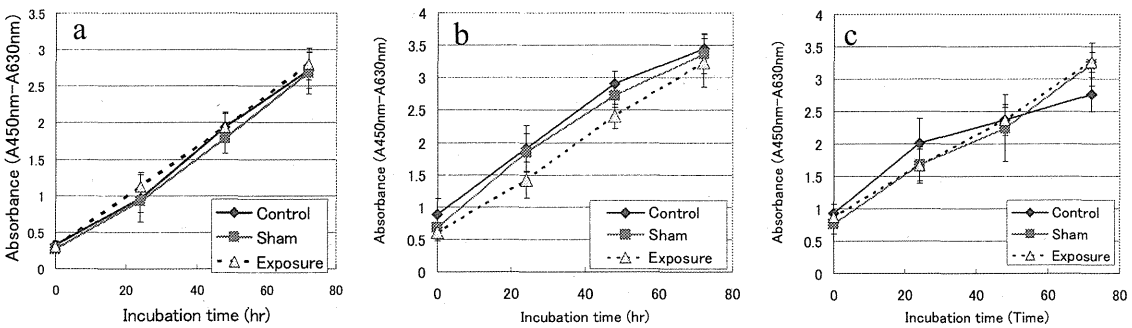

Fig. 2 Cell growth under IF-MFs (a; 2mT, b; 3mT, c; 3.9mT)

In *in vitro* micronucleus assay, micronucleus frequency in CHL/IU cells was not affected and remained almost same as sham control by exposure to 2mT, 21kHz IF-MF for 24 hr. On the other hand, treatment with MMC (0.04 μg/ml) caused significant increase in micronucleus frequency. These results are shown in Fig. 4. Accordingly, it indicated up to 3.9mT, 21kHz IF-MF did not induce micronucleus in CHL/IU, cultured mammalian cell line.

Dosimtry of each sample was estimated by analytical solution method. As the result, average induced currents were estimated 6.33 A/m^2 in 90 mm dish, 4.22 A/m^2 in 60 mm dish and 0.05 A/m^2 in 96 well microtiter plate under 21 kHz, 2mT IF-MFs exposure. This estimation suggests that induced current did not affect cell growth and micronucleus frequency in culture condition even in the level was exceeding the basic restriction of ICNIRP guideline in 1998.

Previously, lack of genotoxicity of IF-MFs (20 kHz, up to 1.1 mT for long term exposure [4], and 23 kHz, 6.05 mT for short term exposure [5]) have already been reported. Our results confirm these results in part and also indicate long term exposure to strong IF-MF did not induce mutation and also toxic effect in mammalian cells.

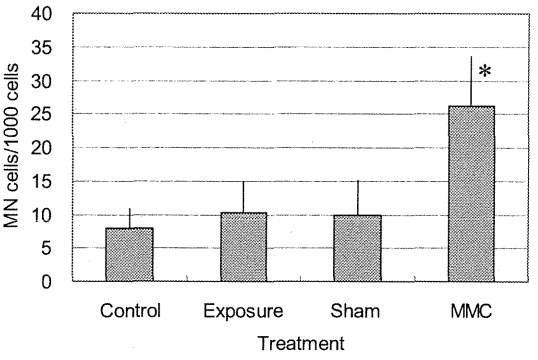

Fig. 4 Micronucleus formation per 1000 cells in CHL/IU cells by exposure to 2mT, 21kHz, IF-MF. (Control; conventional CO_2 incubator, Exposure; 2mT, 21kHz IF-MF, Sham; sham exposure, MMC; 0.04 μg/ml of mitomycin C, *; $p<0.05$ by Student's *t*-test)

4. Conclusion

We developed an exposure system for *in vitro* test systems, such as cell culture, to expose to strong IF-MF (21kHz, up to 3.9 mT) continuously. Using this system, we demonstrated that at least 3.9mT, 21kHz IF-MF (that is 144 times higher than current ICNIRP reference level for public) did not have toxic effects in mammalian cell lines and also did not induce micronucleus. This suggests IF-MFs in environment is unlikely to have adverse health effects.

5. Acknowledgement

This work was supported in part by The Ministry of Health Labour and Welfare in Japan, Health Labour Sciences Research Grant (08150668).

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5. S. Nakasono, M. Ikehata, M. Dateki, S. Yoshie, T. Shigemitsu and T. Negishi, "Intermediate frequency magnetic fields do not have mutagenic co-mutagenic or gene conversion potentials in microbial genotoxicity tests", *Mut. Res.*, Vol. 649, pp. 187-200, 2008

Estimation of Mutagenic Effects of Intermediate Frequency Magnetic Field using Mammalian Cells

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Abstract

Since the opportunities that people are exposed to intermediate frequency (IF) magnetic fields (MF) are increasing, the health risk assessment of IF-MF has now become important. Because there have been few studies about long-term exposure to IF-MF with high magnetic flux density, we have developed a new apparatus capable of IF-MF exposure up to 3.9 mT for *in vitro* study. In this study, we found out that IF-MF did not affect both cell growth and mutagenicity using the mammalian cell line CHO-K1 and its DNA repair deficient derivatives.

1. Introduction

In recent years, the opportunities that people are exposed to intermediate frequency (IF) magnetic fields (MF) have been increasing through staying close to such devices as IH cooker, inverter installed on electric train and so on, bringing about the public concerns for IF-MF. However, only a few studies on biological effects of IF-MF have been reported to date, even though there have been a lot of studies related to extremely low frequency (ELF)-MF and radiofrequency (RF)-electromagnetic fields (EMF), reflecting social anxiety about explosive spread of mobile telephony and so on. In such a situation, the health risk assessment of IF-MF has now become important. The World Health Organization (WHO) also recommended that more research to reveal scientific evidence for health risk of IF-MF should be conducted [1]. Since there have been few reports about exposure to IF-MF with high magnetic flux density, especially long term exposure, it is important to evaluate biological effect of long-term IF-MF exposure. Therefore, we have newly developed an exposure apparatus of IF-MF, which is designed for long-term exposure for *in vitro* study [2].

In this study, we investigated the effect of IF-MF on growth rate of various cell lines which were deficient in DNA repair using newly developed IF-MF exposure apparatus. Additionally, mutagenicity of IF-MF was assessed by *HPRT* mutation assay using the CHO-K1 cells, because mutagenicity, which is a part of initiation step of cancer development, is one of the important indices to evaluate the health risk of IF-MF.

2. Materials and Methods

2.1 Exposure Devices

Figure 1 shows the exposure apparatus of IF-MF. This exposure apparatus employed a meritt type coil as a basic structure [2], which can generate up to 3.9 mT homogeneous IF-MF (±5%), at the frequency of around 20 kHz in the cubic space of 150 mm on a side. A CO_2 incubator was installed in that homogeneous IF-MF space to be able to expose MF to cultured cells at 37±1°C under 5% CO_2 conditions. We made two identical apparatus combined a CO_2 incubator and a merit coil were made and used with eneregizing (exposure) or without energizing (sham exposure: no-magnetic field) simultaneously. In this study, IF-MF exposure condition was set to be 21 kHz, 2, 3 and 3.9 mT for from 24 to 72 hrs.

Fig. 1 The exposure apparatus for IF-MF exposure.

2.2 Cell Growth

Chinese hamster ovary cells, the CHO-K1 and its DNA repair deficient derivatives, xrs5 (*Ku86*), V3 (*DNA-PK*), irs1SF (*XRCC3*) and EM9 (*XRCC1*) were used in this study. The CHO-K1 was incubated in the Ham's F12 medium and other cell lines were the alpha minimum essential medium. To investigate the growth rates, 100 μl aliquot of pre-cultured these cell lines (10^4 cells/ml) was poured into each well of 96-well plates and incubated for 24 hrs prior to the exposure period, then the plates were exposed to sham or IF-MF (2 or 3.9 mT). After 0, 24, 48 and 72 hrs, 10 μl of the WST-1 premix solution (TaKaRa Bio Inc.) was added to 4 well each and then put back to the apparatus. The WST-1 method is colorimetric assay based on cleavage of the tetrazolium salt WST-1 by mitochondrial dehydrogenases in viable cells. Absorbance at 450 and 630 (for reference) nm was measured using microplate reader (Bio-Rad laboratories, Inc.) after incubation for 3 hrs.

2.3 Mutation Assay

In this study, *HPRT* mutation assay was done using the CHO-K1 cell line. The CHO-K1 cells (1.25×10^5 cells) in 60 mm culture plate were pre-cultured for 24hrs prior to the exposure period, and then exposed to sham or IF-MF (2, 3 or 3.9 mT) for 24hrs. As a positive control, cells were added to culture medium including 5 μg/ml of methyl methanesulfonate (MMS) and incubated for 24 hrs. After exposure, medium was changed to new medium and cells were incubated another 24 hrs. Exposed cells were subcultured once a several days for 5 days to fix mutation. After 5 days, these cells were subcultured in the Ham's F12 medium for cloning efficiency and the medium containing 6-thioguanine (TG) for 6-TG resistant mutant detection. Colonies were counted after 6 and 10 days for survival cells and 6-TG resistant cells, respectively. Based on these data, mutation frequency was calculated as 6-TG resistant cells per 10^6 survival cells.

3. Results and Discussion

3.1 Cell Growth

Figure 2 shows the results of cell growth using the WST-1 method in the CHO-K1 cell lines and its DNA repair deficient derivatives exposed to 2 mT IF-MF. Comparing growth rate between sham and IF-MF exposures, there was no statistically significant difference in cell growth of any of the cell lines used in this study for up to 72 hrs-exposure. For 3.9 mT IF-MF, no significant differences were also observed (Data not shown). These results suggested IF-MF exposure does not affect cell growth regardless of lack of the ability of DNA repair, although some studies relating static magnetic field or ELF-MF suggested some effects on DNA repair deficient organisms or cells [3, 4]. The cell lines used in this study were deficient in genes related to repair of single strand break or double strand break such as non-homologous end-joining and homologous recombination. Therefore, these results suggested that IF-MF did not affect the repair pathway that was involved these genes at least.

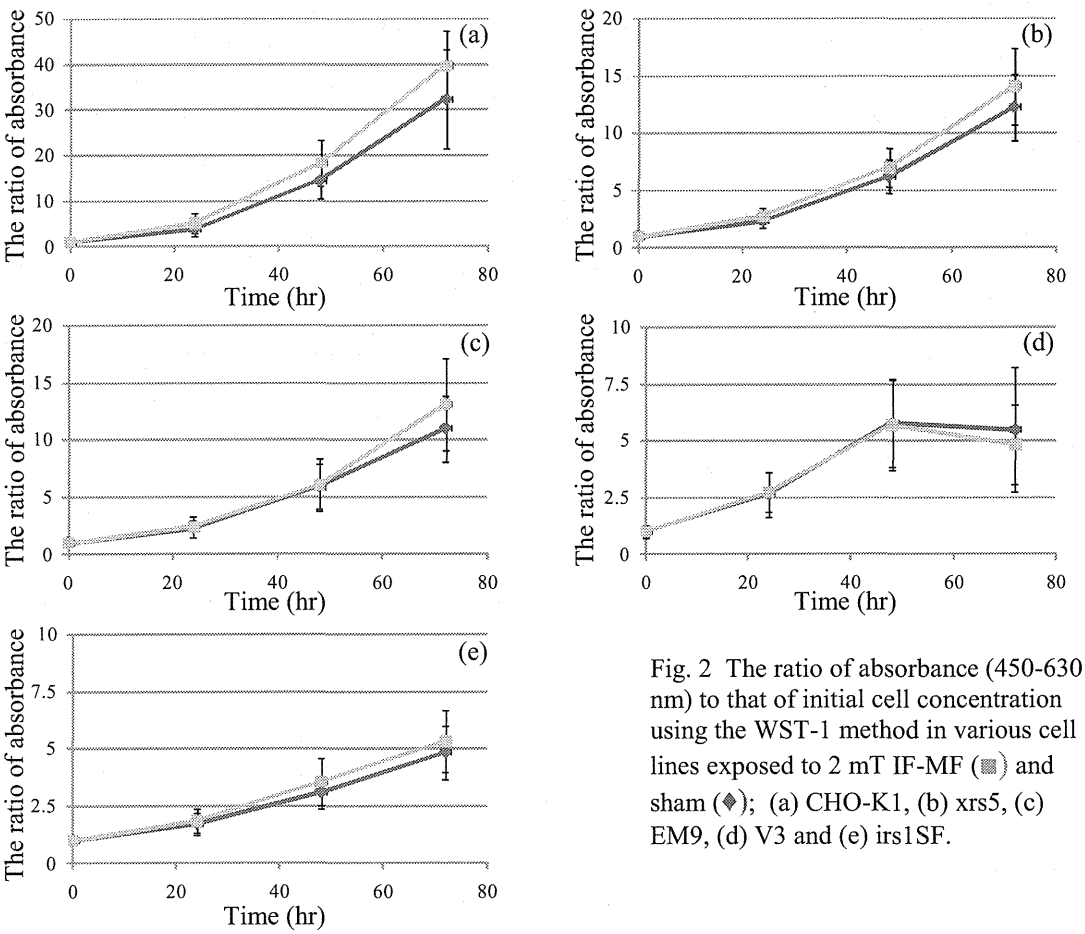

Fig. 2 The ratio of absorbance (450-630 nm) to that of initial cell concentration using the WST-1 method in various cell lines exposed to 2 mT IF-MF (■) and sham (◆); (a) CHO-K1, (b) xrs5, (c) EM9, (d) V3 and (e) irs1SF.

3.2 Mutation Assay

Figure 3 shows the ratio of *HPRT* mutation frequencies in the CHO-K1 cell line exposed to 2 mT IF-MF to sham exposure. As the result of mutation assay, there were no significant differences between sham and 2 mT IF-MF exposures. In contrast, MMS (5 μg/ml) as a positive control significantly induced 6-TG resistant mutant. For 3 or 3.9 mT of IF-MF, no statistically significant differences were observed between sham and IF-MF exposures. Previous studies about IF-MF exposures under both short-term/high magnetic flux density (23kHz, 2 hrs, 6.05 mT for mammalian cells) [5] and long-term/low magnetic flux density (2, 20 or 60 kHz, 48hrs, 1.1 mT for bacterial cells) [6] conditions showed that IF-MF did not have mutagenicity. The results in this study indicated that IF-MF exposure under long-term/high magnetic flux density condition did not cause mutation also.

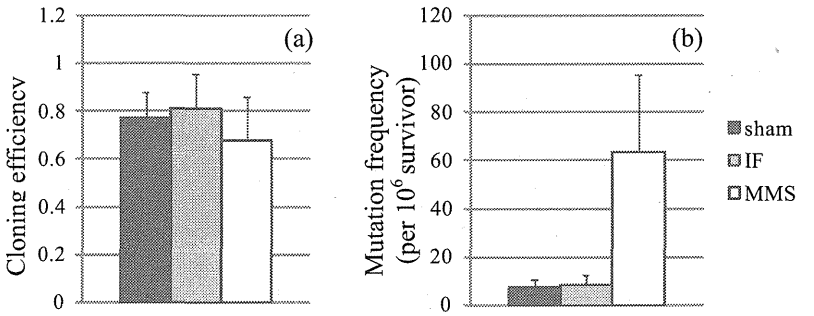

Fig. 3 The result of mutation assay in the CHO-K1 exposed to 2 mT IF-MF; (a) cloning efficiency, (b) mutation frequency.

The magnetic flux density of IF-MF in this study was more than 100 times reference level of international commission of non-ionizing radiation protection (ICNIRP) [7]. Since the magnetic flux density, which was exposed in the general environment, is lower than that exposed in this study, it is indicated that IF-MF generated in the general environment did not have the effect on cell growth and mutagenicity.

4. Conclusion

In this study, long-term IF-MF exposure with high magnetic flux density was conducted using a newly developed exposure apparatus for *in vitro* to investigate biological effects of the IF-MF. The effect on cell growth and mutagenicity of the IF-MF (21 kHz, up to 3.9 mT) was evaluated using the mammalian cell lines CHO-K1 and its DNA repair deficient derivatives. The results indicated that there were no significant differences in cell growth of any cell lines (CHO-K1, xrs5, EM9, V3 and irs1SF) between sham and IF-MF (2 or 3.9 mT) exposures for 72 hrs, suggesting that IF-MF did not affect cell growth regardless of lack of DNA repair ability. Moreover, *HPRT* mutation assay using the CHO-K1 cell line indicated that 2, 3 and 3.9 mT IF-MF exposure for 24 hrs did not have mutagenicity. These results suggested that the IF-MF to which general public was exposed through IH cooktop, electric railway environment and so on would not have mutagenicity and cytotoxicity.

5. Acknowledgments

This work was supported in part by Research on Health Security Control, Health and Labour Sciences Research Grants in Japan.

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Effects of exposure to intermediate frequency magnetic fields on gene expression of estrogen-regulated gene in MCF-7 cells

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Abstract

To evaluate biological effects of intermediate frequency magnetic fields (IF-MF), estrogen-regulated gene expression under magnetic fields were studied. Genetically modified MCF-7 cells that transformed with ERE-luc fusion gene was used. Cells which endogenous estrogen depleted by estrogen free media are exposed to 21 kHz IF-MF for 24 hr. Then, luciferase activity was measured as estrogen-regulated gene expression.

In this study, we have observed no significant difference in luciferase activity between exposed and sham-exposed cells by exposure to up to 3.9mT, 21 kHz IF-MF for 24hr. These results suggest that IF-MF is unlikely to affect directly on estrogen-regulated gene expression.

1. Introduction

Recently, IH hobs are becoming popular and production of IH hobs is over a million units per year in Japan. The IH hobs are very effective and safe to cook because of using IF-MF, not flame. On the other hand, the fact remains that people concern about health risk of IF-MF because assessment of health risk is not enough to date.

To respond this, the World Health Organization (WHO) is conducting the International EMF Project to evaluate possible health risk by exposure to electromagnetic fields (EMF). WHO already published two Environmental Health Criteria monographs for static fields (No. 232, in 2006) [1] and time-varying (<100 kHz) extremely low frequency fields (No. 238, in 2007)[2]. However, health risk of IF-MF is inconclusive because there is few researches to date, thus further research in frequencies between 300 Hz to 100 kHz are recommended by WHO.

To evaluate a possible health risk of IF-MF, it is important to investigate various biological effects of exposure to IF-MFs. In case of using IH cooking hob, effect on the pregnant woman and the fetus should be carefully investigated in addition to evaluate its carcinogenic and toxic effect. In this study, estrogen-regulated gene expression under IF-MFS was studied in order to evaluate the effect on women who are considered as a main user of the IH hobs.

2. Materials and Methods

Genetically modified MCF-7 cells (human breast adenocarcinoma cell line) that were transfected with the ERE-Trans Lucent Reporter Vector (Panomics) using Lipofectamine were used in this study. As the result of transfection, EREs-luc fusion gene (Estrogen Response Elements with luciferase gene) was translocated on genome of the cells. Thus, estrogen induces expression of the luciferase in these cells.

For IF-MFs exposure, an original exposure system that is capable of generating 21 kHz, IF-MF up to 3.9mT (144 times higher than reference level for public in the ICNIRP guideline published 2010) within exposure space (150mm×150mm×150mm) within ± 5% deviation was used [3].

Pre-cultured cells are re-inoculated in estradiol free media (phenol red free MEM media with insulin and activated charcoal treated FBS) for three days to deprive cellular estradiol. After this treatment, cells were harvested and re-suspend in estradiol free media. Then, cell aliquot was divided three 6-well plates. One is for IF-MF exposure, second group is for sham exposure and third group for control. In each plate, three wells contained 10^{-11} M estradiol. For exposure period, exposure group incubated in exposure device at 0, 2, 3 and 3.9mT with 21 kHz IF-MF, respectively, while sham exposure group incubated in identical exposure device

without power unit. Control group incubated in a conventional CO_2 incubator.

After exposure to IF-MF for 24 hrs, cells were harvested and rough total protein solution was collected using cell culture lysis reagent (Promega Co., U.S.A.) on ice. The extract was directly used for chemiluminescence assay using Luciferase assay kit (Promega Co., U.S.A) and for quantification of total protein concentration by Lowry method using Bio-Rad DC protein Assay Kit (Bio-Rad, U.S.A.). Measurement of chemiluminescence was performed by manufacturer's protocol using chemiluminescence meter (Lumat LB 9507, Berthold Technologies, U.S.A).

3. Results and Discussion

Because of its high sensitivity and uncertainty, there is large deviation of measurement data in chemiluminescence over experiments. Therefore, analysis of the data was performed in each experiment with three wells per treatment, not inter experiments. Fig. 1 shows a typical result of an experiment of 0mT IF exposure (sham exposure), sham exposure and an incubator control. There is no significant difference between sham and sham with/without 10^{-11}M estradiol. This indicates both identical exposure device provides almost same cultivate environment for MCF-7 cells. We performed two to four independent tests in sham-sham, 2mT-sham, 3mT-sham and 3.9mT-sham condition. Fig. 2 shows a typical result of an experiment of 2mT-sham and an incubator control. It is almost similar to sham-sham experiment and there is no significant difference among an incubator control, sham and 2mT with/without 10^{-11}M estradiol. In 3mT-sham and 3.9mT-sham experiments, no significant differences were also observed (Data not shown). These results indicate that exposure to 21 kHz, up to 3.9mT IF-MF for 24 hrs did not have potential to induce estrogen regulated gene expression on the estimation of our reporter gene assay.

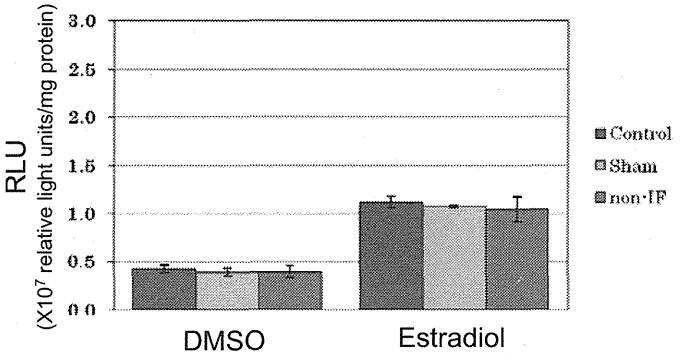

Fig. 1 Result of luciferase assay by IF (0mT:sham) and sham exposure.

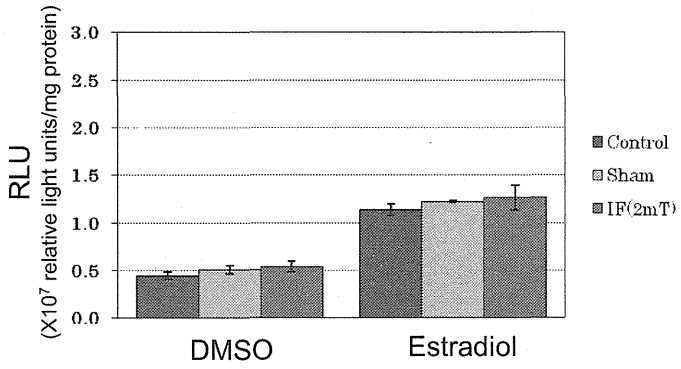

Fig. 2 Result of luciferase assay by IF (2mT) and sham exposure.

4. Conclusion

To evaluate biological effects of intermediate frequency magnetic fields (IF-MF), estrogen-regulated gene expression under magnetic fields were studied. From the results presented here, we have not found any significant difference in luciferase activity between exposed and sham-exposed cells by exposure from 2.0 to 3.9 mT, 21 kHz IF-MF at least for 24hr. To evaluate the effect of IF-MF on the pregnant woman and the fetus in detail, we will investigate the effect of longer period of exposure to IF-MF on the expression of estrogen-regulated gene in MCF-7 cells in further study.

5. Acknowledgement

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Evaluation of Biological Effects of Magnetic Fields -from static to intermediate frequency-

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Abstract

Mutagenic potential of various magnetic fields were investigated. Slight mutagenic and co-mutagenic potential were observed in strong static magnetic field above 2T while lack of effect found in other exposure conditions. This finding was slight effective on anti-tumor treatment in L1210 bearing BDF1 mice as a possible application in medicine.

Keywords : magnetic fields, biological effects, mutagenicity, medical application

1. Introduction

Progress of technology is capable to use electric, magnetic and electromagnetic field (EMF) in various purposes. For example, static magnetic field (SMF) are used for levitation and propulsion of MagLev train car, SMF and intermediate frequency (IF) magnetic fields are used for magnetic resonance imaging(MRI), several tens kHz intermediate frequency (IF) magnetic fields are used for induction heating of cooking pan, etc. Also, many electrical devices generate various magnetic fields in results of using electric current as their power source, such as high-power inverter for electric train. These technologies bring us large benefits, however it is also necessary to evaluate its health risk to avoid confusion in society because of lack of possible health effect of EMF. Recently, WHO published two environmental health criteria monographs for static fields (No. 232, in 2006) [1] and time-varying (below 100 kHz) extremely low frequency fields (No. 238, in 2007) [2]. In these monograph, lack of basic knowledge was still pointed out. On the other hand, if biological effects of MFs exist, study of application in medicine should be promoted.

2. Materials and Methods

2.1 Magnetic field exposure

Superconducting magnet was used to expose up to 5 T (tesla) static magnetic field [3]. Helmholtz's coil was used to expose 50 Hz, up to 40 mT magnetic field [4]. These exposure apparatuses were located in constant temperature room and maintain exposure space at 24, 30 or 37 +/- 1 °C, respectively. Meritt coil was used to expose 21 kHz, up to 3.9 mT magnetic field [5]. Temperature, humidity and concentration of CO_2 was controlled using an incubator that made by resin that was located inside of the coil at 37 +/- 1 °C.

2.2 Mutation assays

For Ames test, *Salmonella typhimurium* TA98 and TA100 were used. Cultured bacterial cells were poured on minimal glucose agar plates with a trace of histidine and biotin. These test plates divided into two groups and one group was incubated under magnetic field while the other group was incubated without magnetic field as control. After 48 hours, revertant colonies were scored.

For yeast mutation assay, 0.1 ml of cell suspension was mixed with molten soft agar (0.6 % Bacto-agar, 0.5 % NaCl) and poured on to low lysine synthetic complete plate for detecting point mutation frequency on *lys 1-1*. 0.1 ml of 1/100 diluted cell suspension was mixed with molten soft agar and poured on to low arginine synthetic complete plate for detecting gene conversion frequency on *ARG4* allele (between *arg 4-4* and *arg 4-17*).

For mouse lymphoma assay (MLA), L5178Y $tk^{+/-}$ 3.7.2C cell was used. Cells were inoculated in a T-25 flask filled with 5 ml of RPMI1640 medium with 10% horse serum ($2.5x10^5$ cells/ml) and were exposed to magnetic field for 48 hr in 5% CO_2 at 37°C with single dilution after 24 hr. Unexposed control cells were incubated in a conventional incubator. After exposure period, plate efficiency and frequency of 5-trifluoro thymidine (5-TFT) resistant cells as $tk^{-/-}$ mutant was determined.

For *in vivo* micronucleus test, BALB/c mice were treated with ascorbic acid at a dose of 200 mg/kg body weight for 20 minutes before injection of doxorubicin (DOX: 6 mg/kg), mitomycin C (MMC: 0.5 mg/kg) or X-ray (0.5-6 Gy). Mice were then immediately exposed to a 5 T SMF for 24 hours. After exposure to the SMF, bone marrow smears were stained with May-Grünwald Giemsa. The number of micronucleated polychromatic erythrocytes in 1000 polychromatic erythrocytes was counted in each animal under a light microscope. Extracted DNA from bone marrow cells was hydrolyzed by nuclease P1 and alkaline phosphatase, and then did ultrafiltration of the hydrolyzate with Microcon YM-10 filter. 8-OHdG concentration was measured by an ELISA Kit.

2.3 SMF Treatment for tumor bearing mice

A passage of the L1210 mouse leukemia cells used a DBA2 mouse strain. Super-conducting magnet was used as a SMFs exposure system. BDF1 mice, transplanted $5x10^6$ of L1210 leukemia cells, were co-exposed to bleomycin (5mg/kg/day for 5 days) and 5 T SMFs until they died. An anti tumour effect of co-exposure to both SMF and bleomycin was estimated by the increase of life span (ILS).

ILS = {(average of life span in exposure group÷ average of life span in control group)-1} x 100 (%)

ILS is an index of beneficial effect of the anti tumour drug (written in the Drug Research and Development, National Cancer Institute (USA)). It is said that ISL is significant in the case of $\geqq$ 25%.

3. Results

3.1 Mutagenicity of magnetic fields

In Ames test, no statistically significant difference in mutation frequency was observed between exposed and control groups in both TA98 and TA100 strain in every experimental condition.

In yeast mutation assay, exposure to a 5 T static magnetic field resulted in a slight but significant increase in gene conversion frequency in *ARG4* locus while reverse mutation in *lys1-1* was not altered in mutagenicity assay (Table 1). On the other hand, no mutagenic effect was observed other experimental condition, such as 50 Hz, 40 mT. This mutagenic effect disappeared on exposure to a 2 T static magnetic field and was even lower than the control on exposure to a 1 T static magnetic field. When cells were exposed to a 0.5 T static magnetic field, there was no difference in the frequency of reverse mutation in both *ARG4* and *lys1*. These results suggest that exposure to a strong static magnetic field shows weak mutagenicity and its threshold would be above 2 T in this tester strain.

Table 1 Mutation frequency by exposure to static or 50 Hz MF in yeast mutation assay.

Treatment		*lys*+ mutants/10^7 survivor (point mutation)		*ARG*+ mutants/10^4 survivor (gene conversion/recombination)	
Control		9.7 ± 2.5[a]		2.4 ± 0.7	
Static, 0.5 T		N.D.[b]		2.1 ± 0.3	
Static, 1 T		10.1 ± 3.0		1.9 ± 0.7	
Static, 2 T		N.D.		2.2 ± 0.1	
Static, 5 T		9.8 ± 3.5		3.3 ± 0.8	*
50Hz, 30 mT		N.D.		2.3 ± 0.5	
50Hz, 40 mT		9.1 ± 2.0		2.7 ± 1.6	
UVB	18 J/m^2	146.1 ± 20.8	*	18.4 ± 3.9	*
	36 J/m^2	311.3 ± 110.3	*	27.4 ± 4.9	*

*Significantly higher than the control group at 1 % level
a) Standard division from at least three independent experiments
b) Not Determined

In the MLA, the plate efficiency that is representative index of acute toxicity was not affected by exposure to all experimental conditions. In addition, the mutation frequency at *tk* allele ($tk^{+/-}$ to $tk^{-/-}$) is almost same between an MF exposed and unexposed cells in all experimental conditions.

In *in vivo* micronucleus test, only exposure to SMF was investigated. The frequency of micronuclei induced by DOX, MMC or X-ray was increased by co-exposure to 5 T SMF, but these increases were inhibited by pretreatment with ascorbic acid. Moreover, 8-OHdG of bone marrow cells was increased significantly after 24 hours exposure to 5 T SMF. 8-OHdG concentration in DNA by irradiation to X-ray (0.5 and 1 Gy) increased significantly after 24 hours co-exposure to 5 T SMF. These increases of 8-OHdG concentration were suppressed by pretreatment of ascorbic acid (data not shown).

3.2 Effect of co-exposure with bleomycin in L1210 bearing BDF1 mice

Mice died within a month since L1210 cells were injected. The ISL was observed 18.5% and 12.3% by exposure to SMF (5 T) and bleomycin (5mg/kg), respectively.

Anti tumour effect of co-exposure to SMF or gradient SMF and bleomycin to the L1210 cells was shown in Fig. 1.The ISL increased to 34.6% by co-exposure to gradient SMFs and bleomycin compared with bleomycin (12.3%) alone groups. Similarly, ISL increased to 27.9% by co-exposure to SMFs and bleomycin. ILS is more than 25%, and an anti tumour effect is expected.

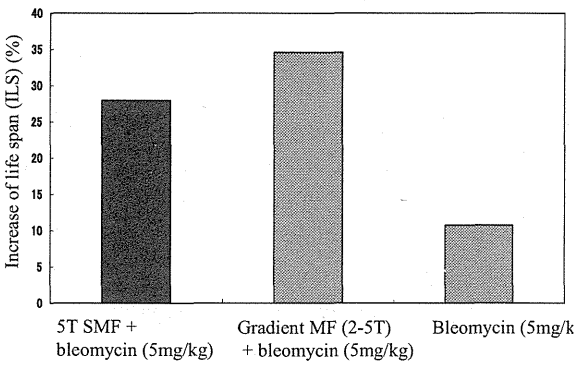

Fig. 1 Antitumor effect of co-exposure to 5T SMF and bleomycin

4. Discussion

In overview of our study, slight mutagenic and co-mutagenic potential were observed in strong static magnetic field above 2 T while lack of mutagenic effect was observed in 50 Hz up to 40 mT, 2 kHz up to 1 mT, 10 kHz up to 1.6 mT and 20 kHz up to 3.9 mT in various mutation assays [3-9]. In static magnetic field, mutation frequency in *Drosophila melanogaster* bearing *mei-41* (human ATM homologue) [3], *Saccharomyces cerevisiae* and *E. coli* bearing *uvrA* with several mutagens [6] and Bulb/c mice [7], was significantly increased by exposure to SMF for 24 to 72 hrs while lack of effect found in SOD deficient *E. coli* [10] up to 13 T. In case of these assays, tester strains have various sensitivity to mutagens and also have different DNA repair abilities. In fact, we found dose response relationship between magnetic field density and mutagenic effect was different among test systems. These different responses, may be depend on DNA repair ability, would be reasonable if the exposure to strong static magnetic field caused an increase of DNA lesion. Although a strong magnetic field even at 5 T obviously does not have enough energy to modify the covalent bond of DNA directly, indirect effects such as increase of oxidative damage by exposure to a strong SMF that reported by Watanabe et al [11]. It is possible this hypothetic effect relate to increase in the mutation frequency that found in our studies. In our study, we found treatment of vitamin E suppressed mutagenic effect by SMF in *Drosophila melanogaster* [3] and this result also supports the hypothesis of indirect oxidative damage by exposure to SMF. On the other hand, the extent of mutagenicity of strong static magnetic field was estimated to be extremely small by comparison of other mutagens. For example, weak UV exposure (18 J/m^2) is approximately 20 times more effective than strong static magnetic field which are at least 10,000 times stronger than those in the environment and long exposure duration (48 hours) in yeast mutation assay, thus this suggests that the effect of static magnetic field even in a 5 T (100,000 times higher than geo-magnetic field) is sufficiently small.

In study of possible application, 5 T SMF or gradient SMF slightly enhanced the anti tumour effect of bleomycin in preliminary experiment. This result suggests that simultaneous treatment of bleomycin and SMF exposure would be effective to leukemia cells such as L1210.

However, it is necessary to evaluate the SMFs exposure condition to improve this possible cancer therapy because ISL of the co-exposure to gradient SMF and bleomycin group was higher than that of the co-exposure to homogeneous SMF (5 T) and bleomycin. To develop this method, it is necessary to apply not only leukemia cell line but also other tumour cell lines such as melanoma, lung cancer, hepatic cancer and so on. In addition, it is important to examine other anti tumour agents, especially free radical producing agent such as adriamycin, X-ray, etc. to screen more effective combination with exposure to SMF because it is known that mode of action of bleomycin is to produce free radicals. In further study, application of antioxidant reagents for this experiment should be investigated to prove a mechanism of anti tumour effect in this test system. Since SMF have already used for clinical diagnosis such as MRI (magnetic resonance imaging), it will not be difficult to introduce this treatment for cancer in future.

In our experience, slight mutagenic effects of MF were found in several test systems but extent of the effects is small even at 5T. It suggests effects of MFs in practical environment such as railway systems, home appliances, etc is extremely small as health risk. However, such small effect would be effective in case of medicine. Therefore, it will be important to investigate both possible biological effect of EMF in the frequency range that have lack of previous knowledge to assess its health risk, and possible application such as treatment of EMF in medicine impartially.

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エストロゲン応答性レポータージーンアッセイを用いた中間周波磁界の生物影響評価

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Evaluation of biological effects by estrogen reporter gene assay

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Abstract

To evaluate biological effects of intermediate frequency magnetic fields (IF-MF), estrogen-regulated gene expression under magnetic fields were studied using genetically modified MCF-7 cells that transformed with ERE-luc fusion gene. As the results, we observed no significant difference between exposed and sham-exposed cells by exposure to up to 3.9mT, 21 kHz IF-MF.

キーワード：中間周波磁界，ホルモン応答，生物影響，遺伝子発現，ルシフェラーゼ，MCF-7 細胞
(Keywords: intermediate frequency magnetic field, hormonal reaction, biological effect, gene expression, luciferase, MCF-7 cell)

1. はじめに

近年、中間周波帯の電磁界を用いる家電の普及が進んでいる。例えば、電磁調理器（IH クッキングヒーター）は、約 20kHz 程度の磁界を利用して、鉄などの調理器具を誘導損による熱により加熱し食材を調理するが、炎が出ないため、やけどや衣服への着火などの事故のリスクが少なく、また構造上調理後の手入れが容易であるなどの利点から普及が進んでいる。また、これら以外にも、盗難防止装置や鉄道車両の高出力インバータなど、数~数十 k Hz の磁界が環境中に発生している。一方で、この周波帯の磁界に関しての健康リスクについては、評価するための科学的根拠が乏しく、2007 年に刊行された世界保健機関（WHO）の健康リスク評価書である環境保健クライテリア Vol. 238（EHC: Environmental Health Criteria）においては以下の通りに記載され、研究の推進が必須とされている。

「この領域のデータが欠落している現状を考慮し、すべてを包括する必要事項として、通常は 300Hz～100kHz（訳注：原文のまま）とされる中間周波電磁界に関する更なる研究が必要である。健康リスク評価に必要とされる知識ベースの極少数しか集まっておらず、既存の研究の多くは結果が一貫していないので、更なる具体化が必要である。健康リスク評価のための十分な IF データベースを構成するための一般的な要件には、曝露評価、疫学研究、ヒト実験室研究、動物および細胞（*in vitro*）研究が含まれる」[1]。

これまでに、中間周波の遺伝毒性に関しては、*in vitro* 研究においてもいくつかの報告がなされ、いずれも遺伝毒性は認められないとの報告[2-4]であるが、IH クッキングヒーターなどの環境中での使用状況を考えた場合、女性（妊娠した女性を含む）が調理する機会が多く、またその際は腹部が調理器に接近するため、中間周波磁界（IF 磁界）曝露による胎児への影響を評価することは重要であると考えられる。エストロゲン（17β-estradiol or E_2）は代表的な女性ホルモンであり、母体や胎児の発生における様々な調節因子として知られている。本研究では、これまでほとんど検討が行われていない女性ホルモン（エストロゲン）による遺伝子発現制御に対する中間周波強磁界曝露の影響について、エストロゲンに応答してルシフェラーゼが発現するレポーター遺伝子を組み込んだ、女性の乳がん由来の樹立細胞を用いた評価系(レポータージーンアッセイ)を用いて検討を行った。

2. 材料と方法

レポータージーンアッセイの原理は、エストロゲンが細胞膜を通過し核内に存在するエストロゲンレセプターに結合してホモ 2 量体の複合体を形成し、その複合体が染色体