THE REPORT OF STUDY

RESULT BY SUBSIDY

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Magnetic Health Science Foundation

CONTENTS

Preface

Creatures, including the human, have passed their life through a long history of evolution in the environment of geomagnetism. The life environment contains not only the geomagnetism, but also the residual magnetism of rocks as well as the magnetic field caused by the electric current of lightning. Possible effects of the magnetic field, as one of the inevitable conditions in the life of the creature, have long attracted attention of many researchers and encouraged their activities. Even though the effects are not so simple to be described as "A causes B", many experiments have been tried without complete understanding of the phenomena, leaving a file of large amount of empirical knowledge.

Today, many techniques and machineries for health and medical care based on the action of magnetic field are observed in several countries. It is also anticipated in Japan, with the situations such as rising-up of medical care expenditures as the background, applications of magnetic field will gradually be felt important. In addition, in recent medical care, not only using the magnetic fields, which are similar to those observed in natural environment, but also artificially produced magnet, very strong field and AC field, are used to induce, enhance or suppress various phenomena in the body, to be applied to diagnosis and therapy.

Based on such a situation, our foundation intends to contribute to the health and medical care of the nation, by encouraging scientific researches and appealing to the society through seminars. It should be noted, however, that the effect of the magnetic field is generated from some basic phenomena interacting with the complex mechanism of the body. The effect can only be clarified by a long-term persistent effort, not by a short-sighted research.

It is a regrettable tendency, however, in the present scientific sector that researchers are mostly interested in acquiring of degree or achieving successful results in a short period, focusing on obvious cause-effect relationship or phenomenon which invites quantitative descriptions. In view of such a tendency, our foundation prefers to support researchers, who persistently attack a particular problem expecting long-term efforts, rather than those who rush into a short-term result.

This report is the faithful summary of researches, which our foundation supported in the fiscal year 2007. It includes a wide range of topics, from basic aspects to practical applications, intending to pave new ways in this area. It is our hope that the report will motivate researchers with similar interests to start communications and contribute to development of magnetic health science.

Director Masao Saito

Magnetic Health Science Foundation

Influence on antitumor effect of static magnetic field

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Purpose

We have reported that co-exposure to static magnetic fields (SMFs) increased the frequency of chromosome aberration induced by some chemicals (include antitumor agents) [1)-4)].

In this study, we investigated the effect of static magnetic fields on antitumor effect of bleomycin in L1210 leukemia bearing mice. To confirm the co-exposure effect, we measured the level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in total DNA as an indicator of oxidative stress assumed by free radicals in the tumor cells.

Methods

A passage of the L1210 mouse leukemia cells (obtained from Health Science Research Resources Bank, Fig. 1-1) used a DBA2 mouse strain. Super-conducting magnet (Toshiba, Fig. 1-2,) 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 SMFs (5 tesla) until they died. An antitumor 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 antitumor drug (written in the Drug Research and Development, National Cancer Institute (USA)). It is said that ISL is significant in the case of ≧ 25%.

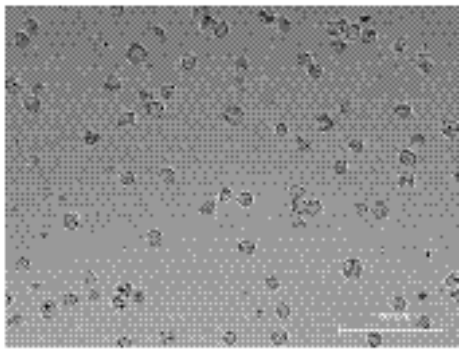

Fig. 1-1 L1210 leukemia cells

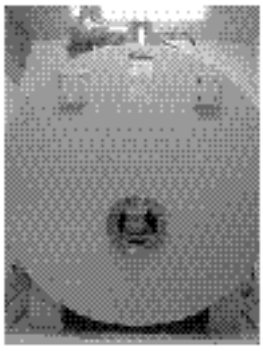

Fig. 1-2 Super-conducting magnet

A mechanism of antitumor effect was determined by the micronucleus inducebility and 8-OHdG in *in vitro* test system.

Results

1. Antitumor effect of SMFs

Fig. 2 shows antitumor effect of SMFs and bleomycin to the L1210 bearing mice, respectively. The ISL showed 18.5% and 12.3% by exposure to SMFs (5T) and bleomycin (5mg/kg), respectively.

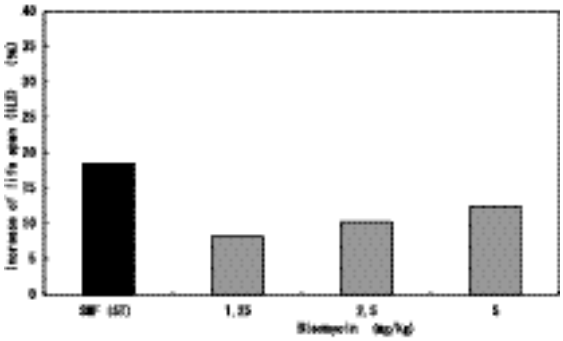

Fig. 2 Antitumor effect ofstatic magnetic fields (SMF) and Bleomycin

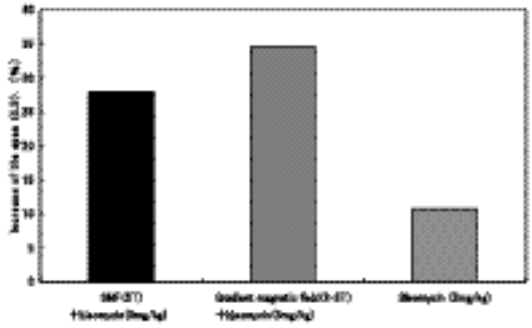

Fig. 3 Antitumor effect of co-exposure to static magnetic fields (SMFs) and bleomycin

Fig. 3 shows antitumor effect of co-exposure to SMFs or gradient SMFs and bleomycin to the L1210 bearing mice, respectively.

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. The As for the co-exposure groups, ILS is more than 25%, and an antitumor effect is expected.

On the other hand, a mechanism of antitumor effect of co-exposure to SMF and bleomycin could not prove by use of the in vitro system.

Discussion

In this experiment, 5T SMFs or gradient SMFs enhanced the antitumor effect of bleomycin. Therefore, simultaneous treatment of bleomycin and SMF exposure may be effective to leukemia cells such as L1210.

However, it is necessary to evaluate the SMFs exposure condition to improve this cancer therapy because ISL of the co-exposure to gradient SMFs and bleomycin group was higher than that of the co-exposure to homogeneous SMFs (5T) and bleomycin. To develop this method, it is necessary to apply not only leukemia cell line but also other timor cell lines such as melanoma, lung cancer, liver cancer and so on. In addition, it is important to examine other antitumor agents, especially free radical producing agent such as adriamycin, X-ray, etc. to screen more effective combination with exposure to SMFs because it is known that bleomycin can induce free radicals.

We could not prove a mechanism of antitumor effect of the co-exposure method by use of the in vitro system. In the future, we apply a antioxidant reagents for this experiment to prove a mechanism in vivo.

It will not be too difficult to apply SMFs to medical treatment of cancer in future because SMFs have already used for clinical diagnosis such as MRI (magnetic resonance imaging). Based on this study, reducing side effects might be possible by reducing the dose of anticancer agents in the future.

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High Gradient Magnetic Separation of Veterinary Antibiotics in Agri-Food Wastes

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Purpose

Antibiotics are widely used to treat animal diseases and improve productivity in livestock farming. In Japan, annual usage of antibiotics for animal medication is larger than that of human and tetracyclines are largest consumption among antibiotics for animal. In recent years, environmental contaminants of antibiotics derived from livestock wastes are reported. Inappropriate use of medications has raised concerns about increased antibiotic resistance. The presences of antibiotics and resistant bacteria are emerging environmental problem. A certain method for treatment of antibiotics from animal wastes is required for the safety of livestock products. In this work, we studied a simple method for magnetic separating of antibiotic tetracyclines with electrocoagulation using iron electrodes as magnetic seeding.

Method

In magnetic seeding process, the sample solution (100ml) included antibiotic (100mg/L) and electrolyte (NaCl, 1.0 g) was electrocoagulated with iron electrodes for 60 sec in a glass beaker. Then 50 mg of Fe_3O_4 (average particle size: 3 μm) was added to 100 ml of electrocoagulated solution and mixed. The neodymium magnet (4,400 gauss) was set under the beaker and left at rest for 5 min. The supernatant was corrected for concentration measurement. The concentration of antibiotics was analyzed by capillary electrophoresis (Agilent G1600).

Results and Discussion

Table 1 shows an experimental result of magnetic separation of antibiotics. The tetracycline antibiotics (oxytetracycline, tetracycline and doxycycline) were removed more than 80 % from sample solution. It is known that high molecular iron chelates are formed with tetracycline antibiotics. It is considered that the electrocoagulation with iron anode generates iron hydroxides and tetracycline-iron complex. The tetracycline-iron complex and magnetite may be adsorbed onto colloidal particle of iron hydroxides. We compared tetracyclines with cefazolin which is classified into cephalosporin antibiotics. In contrast to tetracyclines, the removal efficien-

cy of cefazolin was relatively low. Because cefazolin can hardly be formed iron chelates. It is indicated that the iron complex with antibiotics allows achieving higher removal by magnetic separation. Figure 1 shows removals by magnetic separation of tetracycline antibiotics added to milking parlour wastewater. The wastewater from milking parlour contains manure and cleaning agent. The results indicate that the magnetic separation with electrocoagulation using iron electrodes is effective method for treatment of tetracycline antibiotics in livestock wastewater.

Table1 Magnetic separation of antibiotics by electrochemical magnetic seeding

Class	Substance	Removal (%)	pH (-)	Iron-chelating
Tetracycline	Oxytetracycline	89.3	3.93	+
	Doxycycline	80.3	3.92	+
	Chlortetracycline	78.1	3.88	+
	Tetracycline	93.2	3.89	+
Cephalosporin	Cefdinir	88.0	6.42	+
	Cefazolin	18.7	7.75	

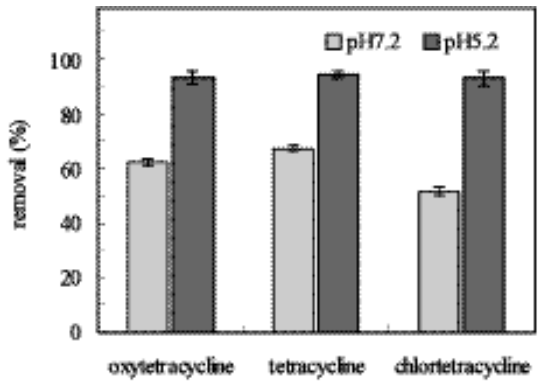

Fig.1 Magnetic separation of tetracycline antibiotics added to milking parlour wastewater

Publication

Ikko IHARA, Kiyohiko Toyoda, Kazutaka Umetsu, Magnetic separation of tetracycline antibiotics with electrocoagulation using iron electrodes, 3rd International Workshop on Materials and Analysis and Processing in Magnetic Fields, p.59 (2008).

I. Ihara, K. Toyoda, N. Beneragama and K. Umetsu, Magnetic separation of antibiotics by electrochemical magnetic seeding, Journal of Physics: Conference Series 156 (2009) 012034

Ikko Ihara, Kiyohiko Toyoda, Mikiko Kita and Kazutaka Umetsu, Magnetic separation of tetracycline antibiotics for treatment of livestock wastewater -Study of the electrochemical magnetic seeding-, JOURNAL of the JAPANESE SOCIETY of AGRICULTURAL MACHINERY, Kansai Branch Report Vol.106, 2009 (*In press*)

Ikko Ihara, Kiyohiko Toyoda, Sachie Kudo and Kazutaka Umetsu, Development of magnetic beads for magnetic separation of veterinary antibiotics, JOURNAL of the JAPANESE SOCIETY of AGRICULTURAL MACHINERY, Kansai Branch Report Vol.106, 2009 (*In press*)

Study on The Mechanism of Vaso-relaxation by Specially Arranged Magnetic Device

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Purpose

It has been well documented that many Japanese workers often feel strong fatigue for a relatively long time presumably due to engagement to hard work, a potent stressor in our daily life. Hence, it is very important to establish the effective and handy methods to minimize fatigue. Since fatigue is enhanced by over stimulation of a sympathetic nervous system that increases the resistance of arterial network and decreases the effectively circulating blood volume, stimulation of a parasympathetic nervous system that enhances the relaxation of vascular smooth muscle cells and increases blood circulation by enhancing the relaxation of vascular smooth muscles would be important for the reduction of fatigue. It has been well documented that relaxation of arteries is induced by endothelialy generated nitric oxide (NO) that binds to the heme moiety in the active site of guanylate cyclase in smooth muscle cells to decrease cytosolic concentrations of calcium, a major factor for the induction of vaso-relaxation. Since the heme moiety of the enzyme contains paramagnetic iron whose physicochemical properties are affected by magnetic fields, the catalytic activity of the enzyme might be affected by a specially arranged magnetic device. The purpose of the present work is to test the effects of specially arranged magnetic field on the blood flow and elucidate its possible effects on the promotion of human health.

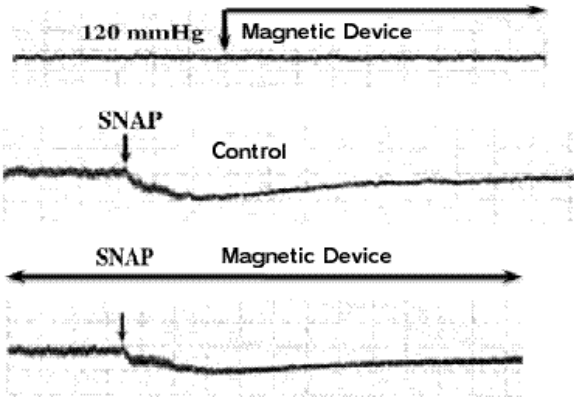
Methods

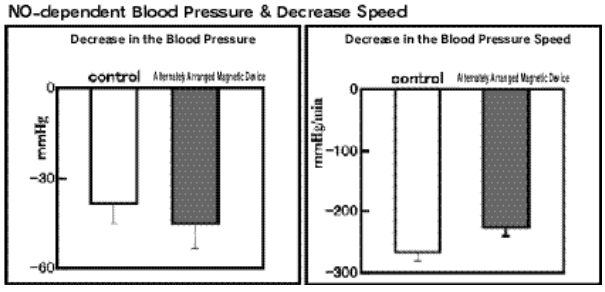
The effect of specially arranged magnetic device on the blood flow of healthy human subjects was analyzed using a high sensitive thermometer. Its effect of the NO-dependent relaxation of the artery was also analyzed in a pentobarbital-anesthetized rat using SNP as an NO donor.

Results

We found that the specially arranged magnetic device focally increased the dermal blood flow of healthy human subjects in a reversible manner as determined by using a high sensitive thermometer. To analyze the mechanism of the increase of blood flow, we tested the effect of

the device on the NO-dependent relaxation of the resistance artery of pentobarbital-anesthetized rats. Although the magnetic device itself showed no appreciable effects on the systemic blood pressure of the rat, it enhanced the SNAP-induced decrease in the blood pressure. The systemic blood pressure of human subjects was also unaffected by the device.





Discussion

The present work showed that the specifically arranged magnetic device reversibly increased the blood flow of healthy human subjects as measured by using a thermometer. Kinetic analysis revealed that the magnetic device enhanced the decrease in the blood pressure of pentobarbital-anesthetized rats that were administered with SNP, a NO-donor. Since SNP undergoes spontaneous degradation to release NO that has high affinity to iron, this observation indicates that some iron-containing molecule(s) involved in the signaling pathway leading tovascular relaxation would be a possible target for the magnetic device. Among various molecules in the artery, guanylate cyclase would be one of the candidates to interact with NO. The magnetic device enhanced the NO-dependent relaxation of smooth muscles in the resistant artery of animals presumably by stimulating the catalytic activity of guanylate cyclase in smooth muscle cells thereby decreasing the cytosolic concentrations of free calcium to enhance muscle relaxation. The findings suggested that the magnetic device increased both dermal blood flow and the depressor effect of NO-donor are consistent with this hypothesis.

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Development of a novel drug delivery system using an organ magnetic material and analysis of its anticancer effect.

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Purpose

The purpose of this study was to characterize the material and to develop a new cancer chemotherapeutic approach using a drug delivery system (DDS).

Methods

Biological characterization of a novel organic magnetic material

Rat prostatic adenocarcinoma R3327-G and mouse lymphoma AJ750 cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum (FBS) and 250 nM of dexamethasone and supplemented with 10% FBS. The cell lines were kindly provided by Dr. J.T. Isaacs (The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins) and by Dr. F. Takeshita (Department of Molecular Biodefense Research, Yokohama City University), respectively. Human melanoma MEL-24 cells (ATCC) were cultured in DMEM supplemented with 10% FBS. All cell lines were cultured at 37°C in a 5% CO_2 incubator. Cells were seeded in 24-well tissue culture plates at 1.5 x 10^4 cells /ml in the presence or absence of a serial concentration of a novel organic magnetic material (OMM)(0-100 μM) for 24 hours, followed by MTT assays and TUNEL staining using a DeadEnd™ Fluorometric TUNEL System (Promega, WI, USA), and nuclei were stained by DAPI (4',6-diamidino-2-phenylindole).

Tumor model animals

LY-54 (Yoshida sarcoma), R3327-MAT-Lu, R3327-AT2.1, R3327-G (rat prostatic adenocarcinomas), MEL-24 (human melanoma), AJ750 (mouse lymphoma) and clone M3 (mouse melanoma) cells were implanted into the tails of Copenhagen rats, Donryu rats and nude mice to observe growth of tumors.

Results

Cytotoxicity by a novel organic magnetic material

R3327-G and AJ750 cell numbers were decreased by the novel OMM, whereas the number of TUNEL-positive cells was increased in a dose-dependent matter. In the case 1.8-30 μM of the OMM, there was a 15% decrease in MEL-24 cells and TUNEL-positive cells were

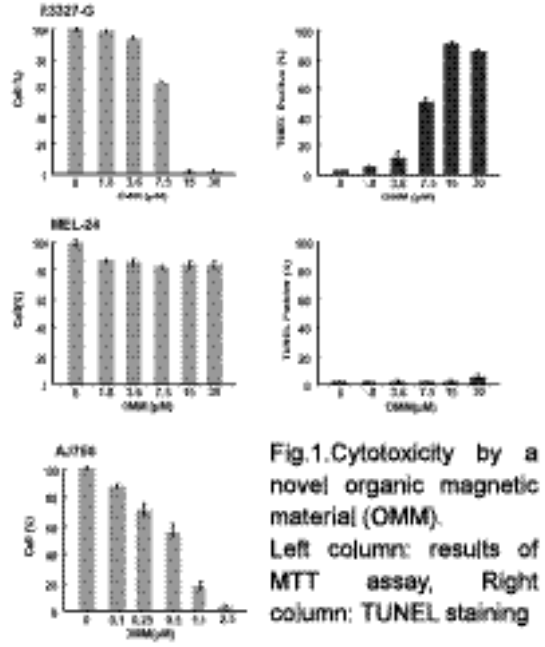

Fig.1.Cytotoxicity by a novel organic magnetic material (OMM). Left column: results of MTT assay, Right column: TUNEL staining

only 5%.

Tumor models

Metastasis was observed in the abdominal cavity 4 weeks after subcutaneous implantation of LY-54 cells in the tail, but the tumor in the tail was not observed at 4 weeks. Tumors of subcutaneous implantations of R3327-AT2.1 and R3327-MAT-Lu cells grew faster in tails of Copenhagen rats. Implantation of melanoma cells into nude mice was also performed, and Clone M3 cells, but not MEL-24 cells, produced melanin pigment. The tumor models are summarized in Table 1.

cell line	Inoculated cell(cells)	host	enlargement (GF)	growth speed	character
LY-54 (s.c) sarcoma	2×10^7	Donryu rat	2	+	metastasis in abdominal cavity
	5×10^7	BALB/c nu/nu mouse	-	-	
R3327-AT2.1 (s.c) prostatic carcinoma	$3\text{-}5\times10^7$	Copenhagen rat	3	2-3	fast growth low sensitivity to OMM
	1×10^6	Rat/Nu/Nu mouse	+	0.3	
R3327-MAT-Lu (s.c) prostatic carcinoma	1×10^7	Copenhagen rat	3	3	fast growth high sensitivity to OMM
	1×10^6	BALB/c nu/nu mouse	3	3	
MeLM3 (human) melanoma	3×10^6	BALB/c nu/nu mouse		3	very slow growth low sensitivity of OMM melanin production
CloneM3 (mouse) melanoma	4.5×10^5	BALB/c nu/nu mouse	4	4-5	high sensitivity to OMM melanin production

Table 1. Tumor models. Cells were subcutaneously implanted in tails. OMM: organic magnetic material

Discussion

We characterized a novel organic magnetic material (OMM) that we isolated and established tumor model animals for development of a material to be used in a DDS.

A cytotoxic effect of the OMM was revealed by biological characterization with MTT assay and TUNEL staining. A DDS has been reported by implantation of LH-54 cells in tails of Holzman rats has been reported[1]), but these rats were unobtainable. Since LY-54 cells have been implanted in Donryu rats, LY-54 cells were implanted in the tails of Donryu rats, but almost all of the tumors metastasized to the abdominal cavity. The development of a more powerful magnet such as a superconducting magnet might be required to deliver the OMM to deep organs, and the subcutaneous implantation was therefore performed. R3327-AT2.1 and R3327-Mut-Lu cells have been implanted into Copenhagen rats [2)3)], but theses rats were also unobtainable. In particular, the tumor model grafted melanoma, clone M3 cells, was useful to estimate growth of the tumor by observation of melanin pigment.

In this study, we revealed that a novel organic magnetic material (OMM) has a cytotoxic effect and we established many tumor implantation models. We are currently attempting to accumulate the OMM in a target tumor by magnetic field exposure *in vivo* (Fig.2).

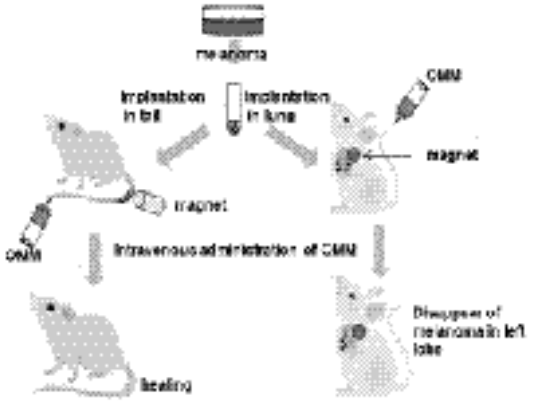

Figure 2. Scheme of the DDS using the OMM in two different modes. Melanoma cells were implanted in the tail or lung.

We expect that results of this study will lead to reduction in the dose of medicine and prevention of severe side effects by enrichment in a

target tumor by magnetic field exposure after administration of a small amount of the OMM or modified OMM.

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MEG differences between eyes-open and eyes-closed resting conditions in schizophrenia

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Purpose

A large number of studies have been performed to elucidate the pathophysiology of SCZ. However, it remains unclear mostly. Recently, a lot of studies for resting brain dysfunction have been conducted using several neuroimaging methodologies [1,2]. Magnetoencephalography (MEG) has been used in the investigation of abnormal neural oscillation which was associated with dysfunction of several cognitive domains in SCZ. To investigate synchronous oscillation in resting state may be leaded to play an important role for elucidation of the pathophysiology in SCZ. However, MEG measurements during only eye-closed resting state might have some possible methodological problems. Therefore, employing the paradigm of eye-open and eye-closed which has several advantage points compared to the paradigm of eye-closed, we investigated synchronous oscillation using MEG. The purpose of our study is to clarify the association between the pathophysiology of SCZ and the change in synchronous oscillation during eye-open and eye-closed resting state. We investigated the change in synchronous oscillation using MEG in SCZ and healthy controls (HC), and analyzed the data applying spatial filter technique which can provide the statistic of group comparison [3]. We hypothesized that event-related synchronization (ERS) in alpha band (8-13Hz) during eye-closed state would be decreased in SCZ compared to HC.

Methods

Twenty-two SCZ and twenty age- and gender-matched HC were enrolled in this study. Their demographic and clinical data were collected. The patient's psychopathology also was assessed. All subjects were less than 60 years old. They were instructed to alternate eye-open and eye-closed state for 10 seconds and to repeat this trial for 8 times. Brain oscillatory changes during eye-open and eye-closed resting state were measured using MEG. Event-related time frequency spectrum applying power value in eye-closed state compared to eye-open state was calculated with the frequency ranging from 1 to 30Hz in 0.5-Hz step (Figure 1). Then the ERS and event-related desynchronization (ERD) in each frequency band were calculated

using Multiple Source Beamformer (MSBF) which was one of the spatial filtering methods. The distribution of ERS and ERD calculated by MSBF were superimposed on the three dimensional image on each subject's brain MRI already co-regsitrated with the standardized Talairach brain (Figure 2). The statistical difference between two groups was analyzed on three dimensional image of the standardized Talairach brain template. This study was approved by the Ethics Committee of Osaka University, and written informed consent was obtained from all subjects prior to the experiments.

Results

As for ERS, the averaged ERS in alpha band during eye-closed state was found in the occipital region dominantly for both groups, but no significant difference was found between two groups. Similar to alpha band, no significant difference for ERS in other bands was found between two groups. However, divided alpha band into two bands (lower alpha; 8-10Hz, upper alpha; 10-13Hz), a significant decrease in ERS in upper alpha band was found in left posterior middle temporal gyrus (PMTG) in SCZ compared to HC (unpaired t-test. d.f. =40, t= 3.551, $p < 0.001$; Figure 3). No significant difference for ERS in lower alpha band was found. No ERD in any bands was found in both groups. Further analysis for upper alpha ERS in SCZ was performed. We investigated the association between the ERS value and the raw scores of subtest of Wechsler Memory Scale-Revised (WMS-R) and between the ERS value and demographic and clinical parameters. A significant positive correlation was found between upper alpha ERS in left posterior MTG and only visual memory subtests of WMS-R in SCZ. No significant correlation between the ERS and any other subtests or demographic and clinical parameters were observed.

Discussion

ERS in upper alpha band is considered to reflect a state of reduced information processing in brain and this idea is consistent with the previous concept of ‘ idling ’ or ‘ nil working ’ [4]. In the present study, a significant decrease in upper alpha ERS was found in left PMTG in SCZ. This finding suggests that SCZ show an reduced idling state in left posterior MTG during from eye-open to eye-closed state. As for correlation with visual memory subtests of WMS-R, these subtest are used in assessing visual recall and saving, and this impairment
appears to be state marker in SCZ [5]. Therefore, the upper alpha ERS in left PMTG during eye-closed state in SCZ might be associated with potential state marker.

In conclusion, we have investigated the change in synchronous oscillation in SCZ and HC using MEG during eye-open and eye-closed resting state. A significant decrease in upper alpha ERS during eye-closed state in left PMTG was found in SCZ. In addition, the ERS value in left PMTG correlated with visual memory which appears to be state effect in SCZ. This finding proposes that the paradigm of during eye-open and eye-closed resting state elicit the change in synchronous oscillation which might represent candidate physiological markers of SCZ., though extensive testing in clinical settings will be necessary.

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Mechanism underlying plasticity induction of the human cerebral cortex by repetitive transcranial magnetic stimulation

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Purpose:

Homeostatic mechanisms have been shown to maintain synapses within a dynamic range of modifiability. A plausible explanation for homeostatic plasticity is the Bienenstock-Cooper-Munro (BCM) theory. The BCM model might directly relate to the experimental observation which revealed the influence of the prior neuronal activity on the magnitude and direction of subsequent synaptic plasticity that is called metaplasticity.

Repetitive transcranial magnetic stimulation (rTMS) has emerged as a promising tool to induce plastic changes that are thought in some cases to reflect N-methyl-D-aspartate sensitive changes in synaptic efficacy. As in animal experiments, there is some evidence that the sign of rTMS-induced plasticity depends on the prior history of cortical activity, conforming to the BCM theory. However, experiments exploring these plastic changes have only examined priming-induced effects on a limited number of rTMS protocols, often using designs in which the priming stimulation alone had a larger effect than the principle conditioning paradigm. The aim of this study was to introduce a new rTMS paradigm (quadripulse stimulation, QPS) that gives a broad range of aftereffects from suppression to facilitation and then test how each of these is affected by a priming protocol that on its own has no effect on motor cortical excitability, as indexed by motor evoked potential (MEP). We also explored the effects of QPS on LTP-like plasticity to shed light on depotentiation mechanism of the human motor cortex.

Methods:

QPS protocols consisted of 360 trains of TMS pulses with inter-train interval (ITI) of 5 s (i.e., 0.2 Hz) for 30 min applied over the hot spot for a hand muscle. Each train consisted of four magnetic pulses separated by a certain inter-stimulus interval. Then, one conditioning consisted of 1440 TMS pulses in total. Cortical changes after QPS were evaluated with MEPs, motor threshold (MT), short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and short-interval intracortical facilitation (SICF). Priming stimulation was performed prior to QPS conditioning. In depotentiation experiments,

long-interval QPS was applied to the motor cortex, after LTP-like plasticity was induced by S-QPS.

Results:

The QPS at short intervals induced a long-lasting MEP facilitation, whereas QPS at long intervals induced a long-lasting suppression. MT which reflects postsynaptic neuronal membrane excitability was unaffected by any QPSs. SICF and ICF were enhanced after QPS at short intervals, whereas SICI remained unchanged. QPS-induced plasticity was altered by priming protocols conforming to the BCM theory: the stimulus-response function of QPS-induced plasticity shifted either leftward or rightward dependent on the priming protocol. As in animal experiments, MEP sizes which were facilitated after short-interval QPS became the baseline level in response to subsequent long-interval QPS protocol.

Discussion:

We have demonstrated a novel and promising rTMS method for inducing bidirectional long-term plasticity of the human motor cortex in a systematic manner. Our investigation provides additional support for the hypothesis that a BCM-like mechanism is at work in the human primary motor cortex. Finally, we showed synaptic depotentiation like plastic changes using two consecutive QPS protocols. QPS will open up a new possibility for understanding learning and memory of the human cerebral cortices and a potential for clinical application.

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Analysis and control of sperm acrosome reactions by high-field NMR spectroscopy

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Purpose

It has been reported that the decrease in sperm motility rates causes severe sterility, while several studies have documented that static magnetic fields can improve the conception rates of cattle sperm. For example, freezing sperm under exposure to magnetic fields [1,2] and control of sperm motility with static magnetic fields [3], have been developed.

Although sperm cannot fertilize the ovum without capacitation and acrosome reaction, it has not been noticed how static magnetic fields influence these factors. The purpose of this study was to investigate the effects of a strong static magnetic field on the motility and acrosome reactivity of mammalian sperm cells, using high-field (14 T) NMR spectroscopy. In addition, we examined the effect of a paramagnetic force on sperm motility under exposure to a strong static magnetic field.

Methods

Human semen samples were collected from healthy volunteers who are not sterile (age group 27-36 years). Samples were placed into conventional 5-mm NMR tubes and spectra were obtained at a frequency of 600.17 MHz using a JEOL JNM ECA-600 spectrometer (14.1 T) in the pulse Fourier transform mode. Chemical shift (δ) and concentration were referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) at 0 ppm.

For paramagnetic orientation experiments, initial spectra were taken and 50 μL of 0.1M $Pr(NO_3)_3$ in D_2O was then added and subsequent spectra taken.

In all of these experiments a pulse width of 13.5 μs and pulse delay of 10 s were used at a temperature of 37°C.

For evaluating the acrosomal status of sperm, we used a double stainning method [4].

Results and Discussion

It is well known that sperm movements are revealed by their flagellar beats. Especially, the movement must be maintained during sperm capacitation and hyperactivation for extended periods. In order to study the effect of a strong static magnetic field on sperm motility, we firstly

evaluated the energy metabolism of sperm with or without exposure to a magnetic field, using NMR spectroscopy.

Figure 1 shows the 600 MHz ^{1}H-^{1}H COSY spectrum of human seminal plasma. In addition, other 2D NMR methods such as the heteronuclear multiple bond correlation (HMBC) and heteronuclear multiple quantum correlation (HMQC) experiments were used to examine the long-range coupling relations to distant functional groups. As shown in Fig. 2, the observed ^{1}H chemical shift values were completely identified and were in good agreement with the reported data [5]. Observably, the methyl resonance of lactate (Lac) revealed a clear signal at 1.3 ppm in the spectra.

Lac is produced from seminal fructose by the metabolism of viable sperm cells. To quantify sperm motility from the variation of NMR spectra, we examined arrayed 1D NMR measurements of semen samples. A quantitative increase in the concentration of seminal Lac was observed, but no significant differences were found in sperm motility as a result of exposure to a high static magnetic field (14 T). Thus the quantification of lactate through high-resolution ^{1}H NMR could be used as a rapid, accurate and relatively simple sperm motility detecting tool. There are two pathways for ATP production in mammalian sperm-glycolysis and mitochondrial respiration. Recently, it has been reported that glycolysis plays a significant role in the energy source of mammalian sperm [6]. This explanation is attractive and is consistent with the Lac peak intensity data described above.

On the other hand, fixed mammalian sperm are oriented with their flat head and tail perpendicular to the magnetic field direction [7,8], because of the diamagnetism of the cell membrane, DNA, and proteins. Under that condition where sperm can move freely, no significant differences were found in the rates of acrosome reactions by NMR spectroscopic analysis or microscopic observation. For the next step, we investigated the possible effects of a paramagnetic force on sperm motility, as illustrated in Fig. 3.

Figure 4 shows the time course of changes in Lac peak intensity on the sperm exposed to a paramagnetic force. When sperm were influenced by a paramagnetic force perpendicular to the magnetic field direction, the energy metabolism due to glycolysis was activated and a tendency for the acrosome reaction to advance was observed. Because lanthanide ions such as Pr^{3+} rigidify biomembranes [9], it was not considered that the sperm acrosome reaction was induced by membrane destabilization. The acrosome reaction advanced depending on ATP exhaustion, because sperm influenced by the paramagnetic force were overloaded with energy for motility.

In conclusion, we succeeded in controlling the acrosome reaction of mammalian sperm influenced by a paramagnetic force under a static magnetic field. Further study, such as the relationships among the paramagnetic force, magnetic field strength and exposure time, is required to optimize the activation process of mammalian sperm under exposure to a magnetic field.

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Presentations

<International>

Hidenori Nakagawa, Shoogo Ueno, Tsuyoshi Shiina, Makoto Kotani, Shunichiro Kubota: Application of Radical Chain Reactions to Drug Release Controlling of Liposomal Carriers Under High Magnetic Fields. 53rd Annual Conference on Magnetism & Magnetic Materials, Austin, USA, November, 2008.

Hidenori Nakagawa, Tsuyoshi Shiina, Wakako Taira, Makoto Kotani, Shoogo Ueno: Effect of Paramagnetic Alignment on the Sperm Motility under High Magnetic Fields. 16th International Conference on Biomagnetism, Sapporo, Japan, August, 2008.

Hidenori Nakagawa, Tsuyoshi Shiina, Makoto Kotani, Shoogo Ueno: Destabilization Effect of Liposomal Nanocarriers Modified with Radical Initiators Studied by Nuclear Magnetic Resonance. 3rd International Workshop on Materials Analysis and Processing in Magnetic Fields, Tokyo, Japan, May, 2008.

<Intranational>

Hidenori Nakagawa, Wakako Taira, Tsuyoshi Shiina, Makoto Kotani, Shoogo Ueno: Analysis of the dynamics of mammalian sperm under high magnetic fields, The 47th Annual Conference of Japanese Society for Medical and Biological Engineering, Kobe, Japan, May, 2008.

Publications

Hidenori Nakagawa, Shoogo Ueno, Tsuyoshi Shiina, Makoto Kotani, Shunichiro Kubota: Application of Radical Chain Reactions to Drug Release Controlling of Liposomal Carriers Under High Magnetic Fields. J. Appl. Phys., 105, 07B323, 2009.

Figure Captions

Fig. 1 Two-dimensional NMR spectrum of seminal plasma (600 MHz).

Fig. 2 Identification of the ^{1}H-NMR peaks with main seminal components.

Fig. 3 Scheme for paramagnetic stress induction to sperm cells.

Fig. 4 Time course of changes in NMR peak intensity of seminal lactate under high magnetic field.

The Subjects for the 2008 Research Grants

Here are the subjects (3 Basic Researches, 2 Application Studies and 2 Specific Researches) that the 2008 Research Grants are subsidized.

Ⅰ, Basic Research

Ⅰ-1. Development of Magnetic Force-Based Tissue Engineering

Kyusyu University / Akira Ito

Ⅰ-2. **Preparation and Evaluation of Ferromagnetic Microcapsules Suitable for Hyperthermia of Cancer**

Kyusyu Institute of Technology／Toshiki Miyazak

Ⅰ-3. Effect of Magnetic field on Circadian Regulation

Tokyo Medical and Dental University, Medical Research Insitute/Jun Hirayama

Ⅱ. Application Study

Ⅱ-1. **Development of a novel drug delivery system using an organ magnetic material and analysis of its anticancer effect**

Department of Otolaryngology, Keio University, University of Medicine/Sho Kanzaki

Ⅱ-2. Development of novel MRI with tumor targeting property

Graduate School of Pharmaceutical Sciences, Osaka University/Yohei Mukai

Ⅲ. Specific Research

Ⅲ-1. Cerebellar influence on spinal motoneurons using peristimulus time histogram

Graduate School of Medicine Sciences, kyusyu University/Ryouhei Ishii

Ⅲ-2. Investigation of the central mechanisms of motor imagery and observation using transcranial magnetic stimulation and clinical application for rehabilitation science

Graduate School of Health Sciences, Hiroshima University/Liang Nan

Note: Affiliations above are at the time of the grants were subsidized.