Evaluation of Damage in DNA Molecules Resulting From Very-Low-Frequency Magnetic Fields by Using Bacterial Mutation Repairing Genetic System

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The effect of very-low-frequency magnetic fields (VLFMF) on living biological cells was investigated using a highly sensitive mutagenesis assay method. A bacterial gene expression system for mutation repair (*umu* system) was used for the sensitive evaluation of damage in DNA molecules. *Salmonella typhimurium* TA1535/pSK1002 were exposed to VLFMF (20 kHz, 600 μT, and 60 kHz, 100 μT) in a specially designed magnetic field exposure chamber. The experimental results showed the possibility of applying the *umu* assay for sensitive and effective evaluation of damage in DNA molecules. No significant difference was observed in the *umu* gene expression intensity under exposure to magnetic field of 20 kHz, 600 μT, and 60 kHz, 100 μT.

***Index Terms*—Mutation repairing gene, *Salmonella typhimurium TA1535/pSK1002*, *umu* assay, very-low-frequency magnetic field (VLFMF), β-galactosidase.**

I. Introduction

ELECTRIC rice cookers, microwave ovens, and induction cooking appliances are widely used in the home. In addition, electric kitchens are being introduced not only into individual houses but also restaurants, school lunch centers, hospitals, and nursing facilities, with induction cooking appliances becoming widely used. Meanwhile, the excitation frequency of induction cooking appliances is being increased from the conventional 20 kHz to about 100 kHz to enhance performance. However, a part of the magnetic field generated by the exciting coils is transmitted to the cooking pan and leaks around outside. As induction cooking appliances become used more widely, the risk to the general public of exposure to very-low-frequency magnetic fields (VLFMF) may have increased [1]. The effect of VLFMF on human health has not yet been completely clarified, but the public is concerned about possible effects [2].

It is well known that mutation in DNA molecules is a direct cause of cancer development, and there is a close relationship between the oncogenesis of biological cells and molecular damage to DNA. When the DNA of a bacterium is damaged, SOS repairing genetic systems such as a bacterial *umu* operon and a *recA* gene system, which try to repair the damage rapidly, are expressed. In this study, we exposed the bacterium *Salmonella typhimurium* TA1535/pSK1002 to VLFMF, and examined the expression intensity of the activity of β-galactosidase, which is located downstream of the *umu* SOS gene operon, using the *umu* assay method with a nonexposure control [4]. This paper describes the results of the *umu* analysis evaluation of damage to DNA molecules caused by VLFMF exposure with different frequencies. According to the study of Miyakoshi's experiment with exposure of MeWo cells derived from human melanoma to a 50 Hz and 400 mT magnetic field proved that the frequency of inducing mutations for HPRT genes increased in proportion to the density of the induction current in an ac magnetic field [3]. The induction current is proportional to f × B. In our experiment, we paid attention to the induction current and set the density at 20 kHz magnetic field of 600 μT and 60 kHz magnetic field of 100 μT so that an induction current similar to that derived under the above conditions (50 Hz and 400 mT) can be obtained.

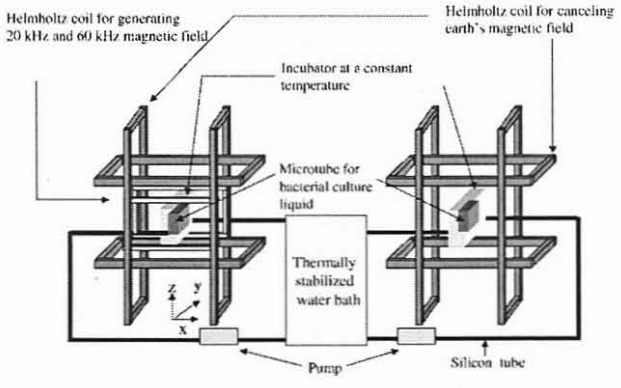

Fig. 1. Experimental system for testing bacteria DNA damage by VLFMF exposure.

II. VLFMF Exposure Equipment

Fig. 1 shows the VLFMF exposure equipment used in this study. It consisted of Helmholtz coils for canceling the earth's magnetic field, a coil for generating the VLFMF (20 kHz, 600 μT, and 60 kHz, 100 μT), and a device for controlling the temperature of a bacterial culture liquid in microtubes. Eight microtubes (3.4 cm in long, 1.1 cm in diameter) with

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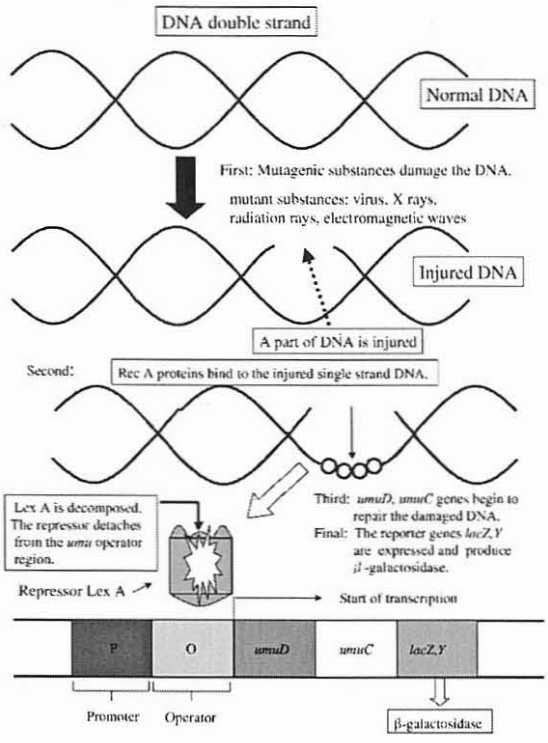

Fig. 2. The principle of β-galactosidase production by damaging a part of the DNA.

the bacterial culture were set in the incubator. The number of bacteria is about 0.15 billion/microtube. To maintain the activity of the bacterial cells, the temperature of the microtube was kept constant at 37 °C. The earth's magnetic field was cancelled by a set of Helmholtz coils. Another set of Helmholtz coils was used for generating the VLFMF. The magnetic field to which the whole bacterial culture liquid in the microtubes was exposed, was 98% uniform. The temperature distribution was uniformly controlled within 37 ± 0.1 °C in whole bacterial culture liquid in the microtubes.

III. Measurements of Activities of β-Galactosidase

S. typhimurium TA1535/pSK1002 is a bacterium that has *umuC, D* and *lacZ, Y* genes. If DNA-destroying substances are present, *S. typhimurium* TA1535/pSK1002 produces β-galactosidase by the gene expression of *lacZ, Y*. The principle of this process is shown in Fig. 2. First, when mutagenic substances damage the DNA in *S. typhimurium* TA1535/psk1002, RecA protein binds to the injured single strand DNA. Then the bound RecA protein decomposes LexA protein, which is a repressor for the *umu* operon. After the repressor detaches from the *umu* operator region, the *umuC, D* genes (ultra mutant genes) begin to repair the damaged DNA. Finally, the *lacZ, Y* genes which are placed downstream of *umuC, D* are also expressed to produce β-galactosidase. Therefore, the degree of damage to the DNA can be evaluated quantitatively, by detecting the activities of β-galactosidase. This is called the *umu* assay method and was used in this study.

The measurement protocol was developed to detect damage in DNA using a mutagenic reagent and VLFMF. The OD_{420} and

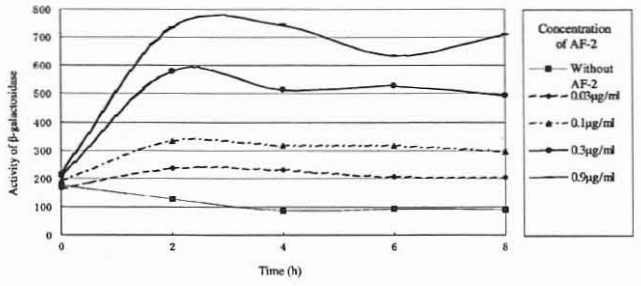

Fig. 3. Activities of β-galactosidase measured with the results of positive control tests and without AF-2.

OD_{550} are optical densities of optical absorbance measured at the wavelengths of 420 and 550 nm, respectively:

$$\text{The degree of activity of } \beta\text{-galactosidase} = 1000(OD_{420} - 1.75 \cdot OD_{550})/(\mathrm{t} \cdot \mathrm{v} \cdot OD_{600}) \quad (1)$$

where "t" is reaction time (in minutes) and "v" is dilution rate.

To confirm the effectiveness of the test protocol developed in this study for DNA damage, furylfuramide (AF2), which is known as a strong mutagen which induces damage in DNA molecules, was used as a positive control. It was clearly recognized that the value of the activity of β-galactosidase increased as AF-2 concentration rose, as shown in Fig. 3. Therefore, the *umu* assay method is effective for examining the influence of environmental factors on DNA molecules.

IV. Measurements of Possible Induction DNA Damage From Exposure to VLFMF

Tables I and II show the results of the β-galactosidase activity of the bacterial culture liquid exposed to VLFMF at the temperature of 37 °C for 2 h and of the control bacterial culture liquid without VLFMF exposure. A Thompson rejection test was performed for each of the eight obtained data. We judged whether the eight sample data could be used and if the data at the time of exceeding a significant level was rejected. The rejected data were shown as "RD" in Tables I and II. The data adopted by Thompson rejection test were equalized, and they authorized whether a significant difference would be between magnetic field exposure and control using t-test at 5% (rate of rejection) of significant levels. Although a significant difference was found in one time measurement (II in Table I), in view of the present results, no significant differences from exposure 20 kHz magnetic field of 600 μT and 60 kHz magnetic field of 100 μT in terms of damage in DNA molecules were observed. Because the range of "Average"±"Standard deviation" of data of the control and the exposure to VLFMF on DNA damage using the *umu* method overlap each other.

Umu-assay using bacteria is an established mutagen test to evaluate the mutagenic capacity of bacteria against genes, and is an official testing method widely used worldwide as a method for testing water supplies and sewage, and for evaluating the safety of drainage. This method has the important advantage of enabling a quantitative evaluation in comparison with other risks by detecting the biological effects and evaluating the safety of

TABLE I
EXPERIMENTAL RESULTS OF DAMAGE CAUSED TO DNA MOLECULES BY EXPOSURE TO VLFMF (20 kHz, 600 μT)

I			II		
Sample number	Activity of β-galactosidase		Sample number	Activity of β-galactosidase	
	Control	Exposure		Control	Exposure
1	253.50	273.11	1	260.70	208.33
2	269.32	261.08	2	259.76	230.35
3	RD	256.77	3	264.61	193.51
4	257.83	283.15	4	248.54	254.68
5	261.56	271.39	5	239.38	RD
6	276.48	265.50	6	244.40	224.71
7	282.00	290.69	7	263.60	215.54
8	263.35	286.90	8	236.06	203.88
Average	266.29	273.57	Average	252.13	218.71
Dispersion	89.43	133.86	Dispersion	114.04	348.31
SD	9.46	11.57	SD	10.68	18.66
significance	×		significance	○	

III			IV		
Sample number	Activity of β-galactosidase		Sample number	Activity of β-galactosidase	
	Control	Exposure		Control	Exposure
1	355.47	RD	1	262.78	RD
2	393.29	342.34	2	RD	300.75
3	323.70	324.36	3	321.84	300.95
4	391.93	350.19	4	328.24	299.81
5	372.40	333.98	5	348.78	280.23
6	337.74	364.46	6	309.25	274.51
7	352.94	354.37	7	290.73	276.42
8	397.51	372.24	8	301.89	288.97
Average	365.62	348.85	Average	309.07	288.81
Dispersion	664.85	240.95	Dispersion	662.56	120.38
SD	25.78	15.52	SD	25.74	10.97
significance	×		significance	×	

SD = Standard deviation
RD = Rejected data by Thompson rejection test

TABLE II
EXPERIMENTAL RESULTS OF DAMAGE CAUSED TO DNA MOLECULES BY EXPOSURE TO VLFMF (60 kHz, 100 μT)

V			VI		
Sample number	Activity of β-galactosidase		Sample number	Activity of β-galactosidase	
	Control	Exposure		Control	Exposure
1	224.05	214.63	1	259.08	249.15
2	224.41	206.56	2	243.74	200.87
3	229.34	221.12	3	253.31	251.11
4	216.45	224.44	4	199.48	194.44
5	221.09	RD	5	256.49	185.87
6	RD	225.49	6	208.00	242.52
7	220.36	233.17	7	190.78	252.37
8	212.79	223.29	8	208.00	248.32
Average	221.21	221.24	Average	227.36	228.08
Dispersion	25.54	62.01	Dispersion	707.74	729.58
SD	5.05	7.87	SD	26.60	27.01
significance	×		significance	×	

VII			VIII		
Sample number	Activity of β-galactosidase		Sample number	Activity of β-galactosidase	
	Control	Exposure		Control	Exposure
1	261.76	311.83	1	300.29	307.60
2	244.67	256.61	2	312.39	297.45
3	271.51	302.06	3	350.69	287.16
4	RD	259.26	4	325.06	328.12
5	279.66	241.95	5	312.61	368.54
6	274.27	313.03	6	312.43	374.07
7	270.38	305.02	7	345.22	324.84
8	234.57	301.81	8	331.01	343.12
Average	262.40	286.45	Average	323.71	328.86
Dispersion	239.21	723.16	Dispersion	271.59	876.95
SD	15.47	26.89	SD	16.48	29.61
significance	×		significance	×	

SD = Standard deviation
RD = Rejected data by Thompson rejection test

VLFMF. The *umu* assay method was applied to evaluate the direct impact of exposure to a VLFMF on DNA damage for the first time in this study. The experimental results showed that the *umu* assay method could be effectively applied to the evaluation of damage in DNA molecules caused by exposure to a VLFMF. But, it is not possible from present knowledge to make a definitive statement about the safety or hazard associated with exposure to VLFMF.

It is necessary to clarify the threshold value of the significance by exposing a stronger and higher frequency magnetic field. Also, it is necessary to develop the new gene expression system by improving the bacteria having the *lucA, B* genes which are placed downstream of *umuC, D*, and are expressed to emit the luminescence. The damage in DNA molecules can be evaluated by measuring the strength of luminescence.

V. CONCLUSION

In this study, the *umu* assay method, which has been used to detect DNA damage, was used to evaluate the direct impact of exposure to VLFMF on DNA destruction. The results did not reveal any damage to DNA molecules caused by exposure to magnetic field of 20 kHz, 600 μT, and 60 kHz, 100 μT.

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