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EFFECTS OF MICROWAVES ON THE COLONY-FORMING CAPACITY OF HAEMOPOIETIC STEM CELLS IN MICE

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Abstract

A suspension of bone marrow cells from femurs of female (CBA×C57Bl)F1 mice was exposed to 2450 MHz CW microwaves in a specially designed waveguide exposure system. The temperature of the suspension rose, during exposure to microwaves, from 20°C to 45°C, and at an interval within 20°C to 45°C the number of haemopoietic stem cells (CFUs) was determined by the spleen exocolony method. The time of exposure of bone marrow cells to each temperature studied was 20 s. Control suspensions of bone marrow cells were exposed to a water bath temperature. There were no significant effects of the CFUs with the water bath temperature, while after exposure to microwaves the number of spleen colonies was elevated with a nadir at the temperature of 37°C. With a microwave-induced increase of the temperature above 41°C the number of CFUs in the bone marrow suspension decreased. The increase in the number of colonies was related to the rise in the seeding rate of the CFUs as well as to a rise in their proliferative activity, while the drop in the number of colonies was influenced also by heat-killing of the CFUs by microwave exposure.

Key words: Microwaves, mice, haemopoietic stem cells.

The effects of microwaves on cells both in vitro and in vivo include chromosome damage (15), depression of phagocytosis (7), increased membrane permeability (1), reduction of the number of lymphocytes in culture (6), and reduction of the number of haemopoietic stem cells formed in vitro in colonies of granulocytes (4) and erythrocytes (14).

Stimulatory effects of microwaves on the haemopoietic stem cells (CFUs) forming spleen colonies have also been demonstrated after exposure of the cells in vitro (9).

In this report we describe the effects of microwaves on murine CFUs exposed in vitro. When the suspension temperature which resulted from microwave absorption reached 37°C, microwaves stimulated the colony-forming ability of CFUs, while at 45°C there was a reduction in the number of colonies formed by microwave-exposed cells.

Material and Methods

The animals. Female mice of the hybrid strain (CBA×C57Bl)F1, 9 to 10 weeks old, were used. The animals were kept in groups of 20 in glass fibre containers, fed with DOS 2Bst pelleted diet and water ad libitum.

Microwave radiation. The source of microwave radiation was a Mikrodiaterm Prema instrument specially modified for biologic experiments (9). The instrument operates at a wavelength of 2450±50 MHz. A radiation intensity of 240 $mW \cdot cm^{-2}$ was used. The bone marrow suspension contained 10^6 cells per ml in a volume of 10 ml. Ringer's solution was placed in a perspex flask at the mouth of the wave duct, with a starting temperature of 20°C. The temperature of the solution was registered during irradiation with a spirit microthermometer located in the flask perpendicular to the mouth of the wave duct. At a radiation intensity of 240 $mW \cdot cm^{-2}$ the suspension increased in temperature from 20°C to 37°C in 3 min and from 20°C to 45°C in 5 min. After irradiation the solution was left at a temperature of 20°C and transplanted within 5 min. During this period the temperature of the suspension fell on average by 1°C.

Water bath. A suspension of bone marrow cells of the same concentration, volume and initial temperature as the microwave irradiated suspension was placed in a water bath in a perspex flask, using a water temperature sufficient to achieve the same rise in temperature as the microwave irradiation. When exposure to both microwaves and water bath was studied the cell suspension was stirred and the final temperature further checked with a high-speed thermometer.

Ionizing radiation. The bone marrow recipients were irradiated with ^{60}Co gamma rays (Chizostat) 2 h before

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transplantation at an exposure rate of 2.15×10^{-4} $C\cdot kg^{-1}\cdot s^{-1}$. The radiation doses used are stated individually for groups of experiments.

Haematologic methods

CFUs count in the cell suspension. The cell suspension was prepared by flushing the femur with Ringer's solution and diluting so that 0.2 ml suspension contained 2×10^5 bone marrow cells. After the exposure to microwaves or the water bath the cells were injected intravenously in isogenous recipients irradiated 2 h previously with a dose of 7.6 Gy. On the ninth day after transplantation the mice were killed by cervical dislocation, the spleen being removed and fixed in Bouin's fluid (13). After 24 h fixation the number of macroscopically visible colonies on the parietal surface of the spleen was counted and the number of colonies per 10^6 bone marrow cells injected was calculated.

The seeding rate in the spleen. This was determined by the re-transplantation method (10). A suspension of bone marrow from experimental and control groups was divided into two parts, the first being used to determine the CFUs concentration in 10^5 nucleated cells. The second part of the suspension was transplanted in the quantity of 2×10^6 cells to primary recipients irradiated with 8.6 Gy. These animals were killed 24 h after transplantation and suspensions from their femoral bone marrow and spleen were secondarily transplanted to recipients irradiated with 7.2 Gy. On the ninth day after transplantation the secondary recipients were killed, the spleens were removed, and the number of macroscopically visible spleen colonies was counted. The number of CFUs injected into primary recipients was calculated from the number of CFUs in their bone marrow and spleen 24 h after injection. The ratio of these two figures gives the seeding rate of spleen and bone marrow with CFUs 24 h after transplantation.

Determination of the proliferation activity of the cells of transplanted bone marrow using ^{125}IUdR incorporation (12). On the fourth day after transplantation of 2×10^5 bone marrow cells each mouse was given 3.7×10^{-7} Bq ^{125}IUdR i.p. in 0.4 ml Ringer's solution. In order to inhibit thymidilate synthesis, fluorodeoxyuridine (10^{-7} mol) was injected i.p. 30 min prior to the ^{125}IUdR administration. Six hours following the injection of ^{125}IUdR, mice were killed and the spleens and femurs removed and placed in 10% formalin buffer solution for 48 h to remove radioactive iodine not incorporated into DNA. Subsequently the organs were dried and the ^{125}IUdR incorporation was determined by a Nuclear Chicago Gamma well counter and expressed as percentage of applied radioactivity.

Results

The irradiation of a suspension of bone marrow cells with microwaves resulted in an increase of the temperature of the medium, which, in a range from 33°C to 40°C, was followed by an elevation of the output of the number of exogenous colonies in the spleens of bone marrow cell recipients (Fig. 1). If the temperature of the medium was above 41°C, the number of colonies formed from bone marrow cells decreased. The nadir of the elevation of the spleen colony number occurred at the temperature of 37°C. The drop in the number of colonies in a temperature range from 37°C to 45°C (Fig. 2) indicated a linear dependence on the temperature that could be described by a regression line $y=33.72-0.72.\ \times$; the correlation coefficient achieved the value of -0.98.

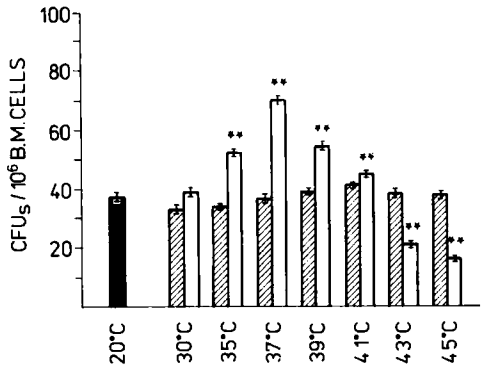

Fig. 1. The number of spleen colonies per 10^6 injected bone marrow cells after microwave irradiation (□) and water bath heating (■). Control group (■). Twenty animals per group. Mean values ± SE. **p<0.01.

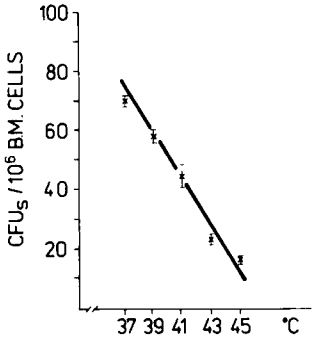

Fig. 2. Dependence between number of CFUs and temperature of suspension after microwave heating.

Heating of the bone marrow cell suspension by a water bath had no effect on the output of the exogenous colony number. The duration of the heating of the suspension by microwaves and by water bath was the same, and the duration of the exposure to the maximum temperature (in each range determined) was not longer than 30 s.

The changes in the seeding rate of the CFUs in the spleen, after transplantation of bone marrow cells, paralleled changes in the number of exogenous colonies (Table) and in ^{125}IUdR incorporation in the spleen.

Table

The effect of short-term heating of bone marrow cells by microwaves (MW) and by a water bath (WB) on the seeding rate of the CFUs and incorporation of ^{125}IUdR into the spleens of bone marrow cell recipients

Group	Seeding rate of CFUs in the spleen (determined 24 h after transplantation)			^{125}IUdR incorporation (determined on the 4th day after transplantation)			
	No. of animals	Per cent of inoculated number	Per cent	No. of animals	Spleen weight (mg)	Incorporation	Per cent
Control 20°C	10	9.2±0.9*	100	12	22.9±1.4	0.074±0.002*	100
MW 37°C	11	22.4±1.6**	243	12	23.2±1.4	0.093±0.003**	126
WB 37°C	9	11.2±1.1	121	11	24.7±1.2	0.078±0.002	105
MW 45°C	10	5.4±0.6**	58.7	10	20.2±0.9	0.054±0.001**	73
WB 45°C	10	8.8±0.7	95.6	12	23.1±1.3	0.070±0.003	95

* versus ** $p<0.01$.

Discussion

The number of exogenous colonies formed in the spleens of recipients of an intravenous inoculum of haemopoietic tissue depends on the number of CFUs applied (5), their ability to seed the spleen's haemopoietic inductive microenvironment (2), and on the humoral influences that induce their proliferation and differentiation (8).

The present study indicates that microwaves are able to change the efficiency of bone marrow CFUs to form macroscopic spleen colonies and that this influence is dependent on the temperature of bone marrow cells heated by microwaves. The elevation of the spleen colony output was in our experiments related to the increased seeding rate of the CFUs in the spleens after heating to 37°C by microwaves, which was followed by an increased haemopoietic activity of the spleen. A study of the mechanism of the stimulating influence of microwaves is under way, and preliminary results indicate that microwaves activate receptors on the CFUs cell membrane which can be blocked by treatment of the suspension with trimepranol and concanavalin A before microwave heating. Such treatments of bone marrow cells eliminate the stimulatory influence of microwaves on the CFUs.

An increase in the temperature of the suspension induced by microwaves from 37°C to 45°C, i.e. by 8°C, resulted in an average drop in the colony-forming efficiency of bone marrow cells by 80 per cent. This drop may be explained as a depression of the seeding rate of CFUs caused by the killing of CFUs by microwaves. The different effect on haemopoietic stem cells, of exposure to microwaves and of warming by a water bath, may be related to the very short time of exposure, and differences in the physical effects of these two sources of heating. While in the case of microwaves there is direct energy absorption by individual components of the cell suspension, in the case of the water bath heat is transmitted only according to the physical gradients of temperature dispersion. It has been shown (11) that in the presence of DNA the microwave absorption in a solution is 24 times higher than in a pure water solution. The fact that heating a bone marrow suspension to 45°C with microwaves causes only incomplete cell death is probably mainly due to the brief exposure to microwaves. It has been found in Ehrlich ascites tumour cells that a temperature of 44°C for 2 h or of 45°C for 30 min causes irreparable DNA damage (3).

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