THE EFFECTS OF HIGH POWER MICROWAVE PULSES ON RED BLOOD CELLS AND THE RELATIONSHIP TO TRANSMEMBRANE THERMAL GRADIENTS

by

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ABSTRACT

Using red blood cells exposed to various pulsed microwave conditions, we examined the possibility that cellular damage may occur due to the creation of microthermal gradients across the cell membrane.

Introduction

Numerous reports have described effects of low level microwave radiation on various cells in vitro, but at the same time, there has been a great deal of difficulty in reproducing the results from one laboratory to another.[1-4] A large part of the difficulty has been attributed to inadequate temperature control in the irradiated sample and also to the complexities involved in determining the absorbed dose of radiation. We have recently designed a compact microwave exposure device which can be readily used to irradiate biological cells at precisely defined microwave conditions while the temperature of the biological material is kept constant ± 0.2°C. The present report will describe data obtained using this device to investigate possible in vitro effects of microwaves on membrane damage to red blood cells. We are especially interested in determining whether or not membrane alterations and subsequent hemolysis could occur during pulsed microwave exposures in which small transient temperature gradients may be induced across the cell membrane. One might expect temperature gradients to occur due to the unequal absorption of microwave power in the cytoplasm versus the extracellular fluid. This concept is of particular importance since Spanner[5] has postulated that a microtemperature gradient of 0.01°C could result in a transmembrane pressure difference greater than 1 atmosphere, and this may be a general mechanism of microwave injury.

Microwave Exposure Conditions

Red blood cells were exposed to pulsed, 2450 MHz microwave radiation using a compact, newly designed, microstrip exposure device.[6] Microwave power was supplied by an EPSCO (model PG5KB) source and a Hewlett-Packard power meter (436A) was used to monitor the power. The pulse duration and pulse repetition rate were measured on a 150 MHz Tektronix DM 44 oscilloscope. Absorbed power was calculated by subtracting the sum of the transmitted and reflected power from the incident power. Using this method of calculation, an incident average power of 1.35 watts resulted in absorbed power of 0.290 watts, or approximately 21%. In terms of specific absorbtion rate (SAR), the 1.35-watt incident power produced an average SAR of 1.75 watts per gram, since the volume of the sample chamber was 0.166 cc and there was 21% absorbtion. A duty cycle of 0.001 was used which gave a peak SAR of 1.75 kilowatts per gram.

Experimental Design

Blood was obtained from rats by cardiac puncture and the red cells were suspended in buffer containing 140 mM NaCl, 25 mM HEPES, 5 mM glucose, and 3% albumin, at pH 7.4. The red blood cell suspension (10 ml) was circulated through the exposure device at a flow rate of 8 ml per minute using a Gilson pump. Temperature was monitored by means of a Yellow Springs thermistor probe placed in line where the blood exited from the exposure compartment. The temperature of the blood was kept between 33 and 35°C by means of a Haake bath and water-jacketed tubing. Using this bath, the temperature did not change more than 0.2°C during the 30-minute exposure period. Samples were removed at 10-minute intervals, centrifuged, and the amount of hemoglobin in the supernatant was determined.[7]

Results and Discussion

A series of microwave exposure conditions was followed which altered the pulse duration and pulse repetition rate to determine whether or not damage may be related to pulse energy. The duty cycle remained the same at 0.001; thus, the 1.35-watt incident power used in these studies gave a peak power of 1350 watts. As shown in Table 1, the pulse repetition rate varied from 16 to 2000 pulses per second (pps), and the pulse duration from 0.5 to 62 microseconds (μsec).

The experimental results comparing hemolysis in control and various microwave exposures are graphed in Figure 1. The protocol consisted of running a series of control-microwave tests on one day and, on a subsequent day, reversing the sequence. The amount of hemoglobin released was determined in duplicate and plotted as a function of time. Data were analyzed by linear regression to determine the best slope. The slope of the microwaved series was divided by that of the corresponding control run on the same day. As shown in Figure 1, the ratios vary above and below 1.0; a value of 1.0 indicating no difference in hemoglobin release between microwaved and control samples. The slope ratio data were used in a paired t-test and no significant differences were found due to microwave radiation. This was true at all microwave exposures tested from 16 to 2000 pulses per second.

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TABLE 1: MICROWAVE EXPOSURE CONDITIONS*

Pulse Parameters, units**	Conditions Tested							
Pulse Repetition Rate, pps	2000	1000	500	250	125	62	32	16
Pulse Duration, μsec	0.5	1.0	2.0	4.0	8.0	16.0	32	64
Energy per Pulse, mJ	0.675	1.35	2.70	5.40	10.8	21.6	43.2	86.4
Absorbed Power, mW	287	293	291	301	283	271	303	-

* Incident power was adjusted to 1.35 Watt and a duty cycle of 0.001 was used in these experiments. Peak power was 1350 Watts. Average absorbed power was 290 mW.

** pps = pulses per second
μsec = microsecond
mJ = milliJoule
mW = milliwatt

There was correspondingly more energy per pulse (86 mJoules) using the 16 pulses per second exposure in which the pulses lasted for 62 μsec. We were especially interested in the longer pulses, with more energy per pulse, because they would create a greater temperature differential if heat could be stored across the membrane. According to Spanner,[5] a temperature of 0.01°C could cause a pressure difference of 1.32 atmospheres across the membrane. Pressure differences even smaller than this may rupture the membrane or alter other membrane functions. The concept is important to investigate because it might be a basic mechanism whereby microwaves interact with biological systems at low average power, but at high peak powers. Therefore, these tests were carried out using microwave pulses with increasing amounts of energy per pulse. At the highest power setting, the EPSCO microwave source was able to deliver enough energy in the 62 μsec pulse to obtain a temperature difference of 0.005°C across the red cell membrane, assuming that all the energy was stored across the membrane. This estimate of the temperature difference is based on dielectric measurements of the buffer-albumin solution and the buffer-albumin cell suspension at 35°C and 2450 MHz radiation.[8,9]

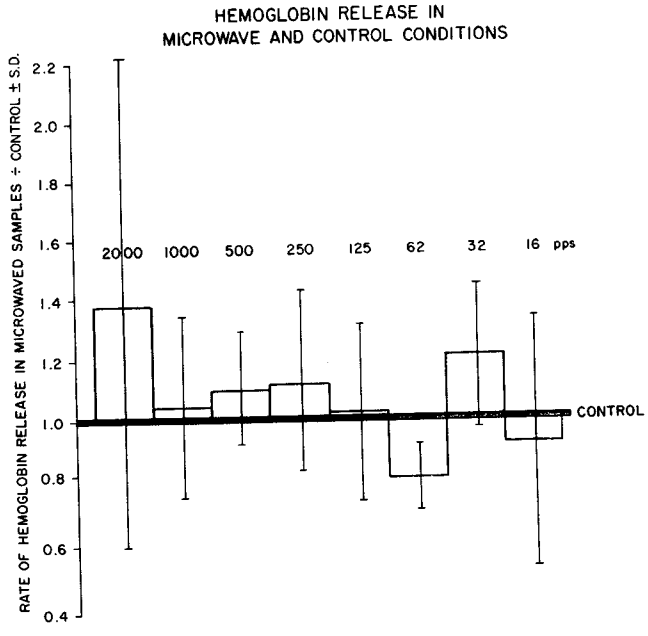

Figure 1. The rate of hemoglobin release from red blood cells was obtained using various microwave exposures from 16 to 2000 pulses per second (pps). This rate of hemoglobin release during microwave exposures was divided by the hemoglobin release under control conditions; thus, the variation around 1.0 indicates either greater hemolysis or less hemolysis, depending upon whether the value is greater than 1.0 or less than 1.0, respectively.

Our analysis of the thermal relaxation time of the gradient across the membrane indicates that the transient part of the gradient dissipates within 0.1 μsec.[10] Thus, heat cannot be stored across the membrane and the longer pulses should not lead to larger transmembrane temperature differences. Experimentally, we saw no increase in hemolysis with increasing pulse length. Despite the short relaxation time, our calculations showed that pulses having a peak power of 1.75 kW/g could cause a temperature difference of about 10^{-4}°C.[8-10] While this may appear to be rather small, it does correspond to a gradient of 100°C/cm across the membrane. A gradient of this size may possibly cause more subtle changes in the membrane and perhaps alter red cell shape or volume.

Currently we are trying to increase the peak microwave power and we are also looking into more sensitive indicators of membrane damage before we dismiss the possibility that transient thermal gradients may damage cells. In summary, we would like to point out that this microstrip exposure device presents a very excellent model to investigate biological effects of microwave radiation, since temperature and microwave power are very easy to determine and reproduce in this system.

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