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Project Summary

Microwaves, Hyperthermia, and Human Leukocyte Function

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Studies were performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. There were no detectable effects on viability or function of human mononuclear leukocytes resulting from exposure to microwave energy at specific absorption rates up to 4 mW/ml. In contrast to studies in other laboratories, results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate insofar as leukocyte function is concerned.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

All individuals are exposed to radiofrequency/microwave energies to variable degrees. Studies by several investigators have raised the possibility that the immunocompetent cells of humans are particularly susceptible to microwaves. These studies were admitted to be poorly reproducible and nonquantitative. Nonetheless, they are frequently cited, and have provided the limited data available, on exposure of human leukocytes, to be used by those individuals and agencies that develop environmental health standards. Many animal systems have been studied, but the species, microwave power intensities, environmental conditions and other factors have been so varied that extrapolation to humans would be exceedingly difficult, even if appropriate.

The studies reported here were performed to determine whether human leukocytes are affected by exposure to microwave energies that equal or even exceed current safety standard recommendations. Exposure to microwave energy at specific absorption rates up to 4 mW/ml resulted in no detectable effects on viability, or unstimulated or stimulated DNA, RNA, total protein or interferon synthesis by human mononuclear leukocytes. In contrast to the earlier studies cited above, results were highly reproducible and provided no evidence that current safety standard recommendations are inappropriate.

Materials and Methods

Human mononuclear leukocytes were exposed in a waveguide system to 2450 MHz (CW) microwaves for 2 hours at specific absorption rates (SARs) from 0.5 to 4 mW/ml. The safety standard limit proposed by Committee C-95.4 of the American National Standards Institute is 0.4 mW/g, which is equivalent in these cultures to 0.4 mW/ml. This standard incorporates a ten-fold safety factor relative to bioeffects reported using animal models. The waveguide exposure system and methods of preventing temperature inhomogeneity, and methods of determining specific absorption rates (SARs) have been described previously (EPA-600/S1-81-041). In addition to leukocyte cultures enclosed within waveguides for exposure or sham-exposure, we included control cultures located within the same incubator but external to the waveguides. No attempt was made to counteract microwave-induced heating of the leukocyte cultures since we wished to observe any potential microwave-induced effects, thermal or otherwise.

Viability was determined by total cell counts and percent of cells able to exclude trypan blue dye and ethidium bromide. DNA, RNA and total protein synthesis were measured by cellular incorporation of tritiated thymidine, urinde, or leucine, respectively, using established methods. Unstimulated and mitogen-stimulated precursor incorporation were assayed from immediately to 5 days after exposure. The mitogen phytohemagglutinin (PHA) was added at a concentration shown to yield optimum responses with normal mononuclear leukocytes, as well as at several suboptimal concentrations. Responses (mean cpm ± S.E.) are shown in *Results* for unstimulated and optimal PHA-stimulated leukocytes.

Results

Exposure of the leukocytes at SAR=4 mW/ml produced no significant changes in cell viability for up to one week after exposure (Table 1). Results were similar with exposures at lower SARs.

Unstimulated and mitogen-stimulated DNA, RNA and total protein synthesis were examined after exposure of the mononuclear leukocytes to microwaves at SARs of 4 mW/ml or less. There were no significant differences between microwave (4mW/ml)-exposed, sham-exposed, and control leukocytes in unstimulated DNA synthesis, or in responses of the leukocytes to an optimal concentration of mitogen (Figure 1). Results were similar using suboptimal concentrations of mitogen, and using lower SARs (0.5 and 1.0 mW/ml) for the microwave-exposed cultures (data not shown). Results were similar with measurements of RNA and total protein synthesis. Microscopic inspection of Wright-Giemsa-stained cytospin preparations did not reveal any discrepancies between morphologic lymphocyte blastogenesis (used in some of the studies cited earlier) and determinations using incorporation of the radiolabelled precursors.

In addition to determinations of total protein synthesis, we measured spontaneous production of interferon (none detected in any cultures), and production of influenza virus-induced interferon-α and PHA-induced interferon-γ, at 1 and 3 days after induction. Virtually all detectable virus-induced interferon-α was present by 24 h, with equivalent amounts produced by microwave-exposed (SAR=4mW/ml), sham-exposed, and control leukocytes (Figure 2A). PHA-induced interferon-γ, usually produced by 48-72 h, was not detected in any culture supernatant fluid at 24 h. By 72 h, interferon-γ was detected in all PHA-induced cultures, with no significant differences between the microwave-exposed (SAR=4mW/ml), sham-exposed, and control leukocytes (Figure 2B). Results were similar with exposures to microwaves at lower SARs.

Table 1. Total Viable Mononuclear Leukocytes after Exposure to Microwave Energy at SAR = 4 mW/ml

	Days After Exposure[a]					
Exposure	1	2	4	5	6	7
Microwave	58±7[b]	60±9	41±4	47±16	39±10	40±10
Sham	65±11	63±15	39±2	72±36	41±9	37±8
Control	54±6	56±6	41±8	46±13	36±7	38±11

[a] *Insufficient numbers of observations (<5) were available 3 days after exposure. Viability was assessed by the ability of the cells to exclude trypan blue dye and ethidium bromide.*
[b] *Data represent mean total number of viable cells (total cells x percent viable) x 10^{-4}, ± S.E.*

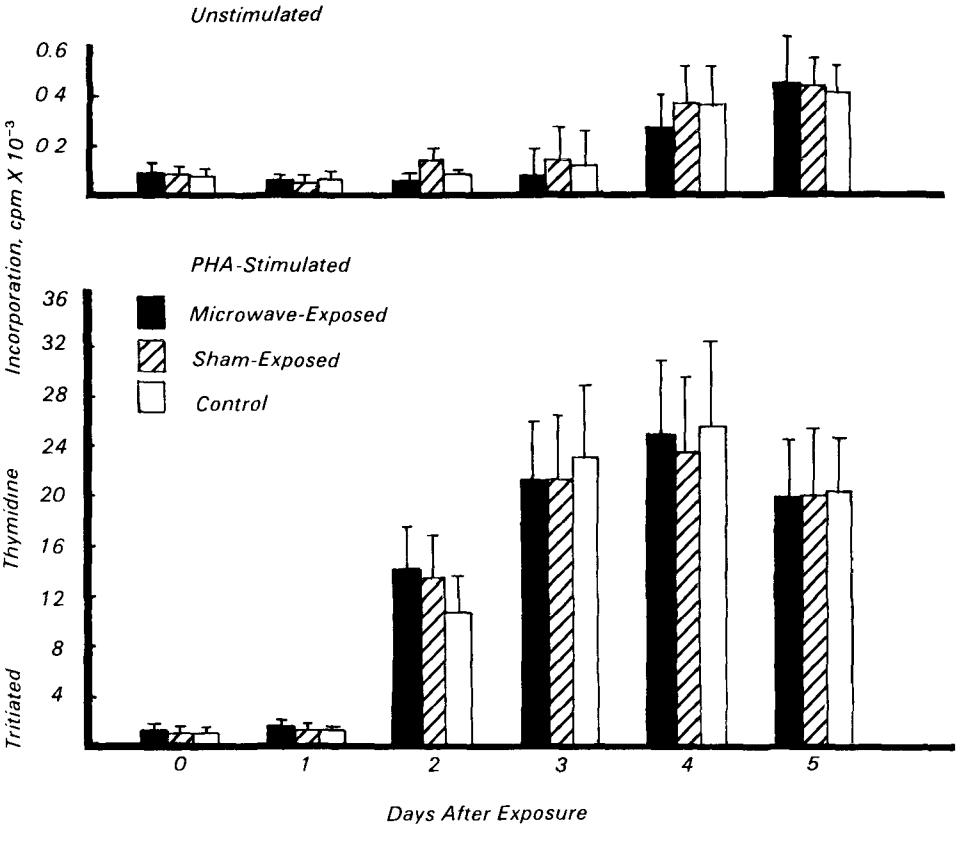

Figure 1. DNA Synthesis by microwave (mW)-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes SAR = 4 mW/ml DNA synthesis by unstimulated cells and by cells stimulated by an optimal concentration of PHA (160 g/ml) are shown. Columns indicate mean cpm tritiated thymidine incorporated ± S.E., from immediately to 5 days after exposure.

Discussion

The current studies provide the first clear, reproducible data regarding exposure of human leukocytes to microwave energies relevant to current public safety recommendations. Direct extrapolation to the *in vivo* setting with many physiological, homeostatic, integrated systems is not appropriate. However, these data do suggest that earlier reports of possible microwave effects on human leukocytes, at such energy levels, remain poorly reproducible and should not form a basis for the resetting of safety standards. Most studies of environmental physical factors examine effects on resting cell populations even though, under normal conditions, man is commonly exposed to more than one environmental stress at a time. Thus,

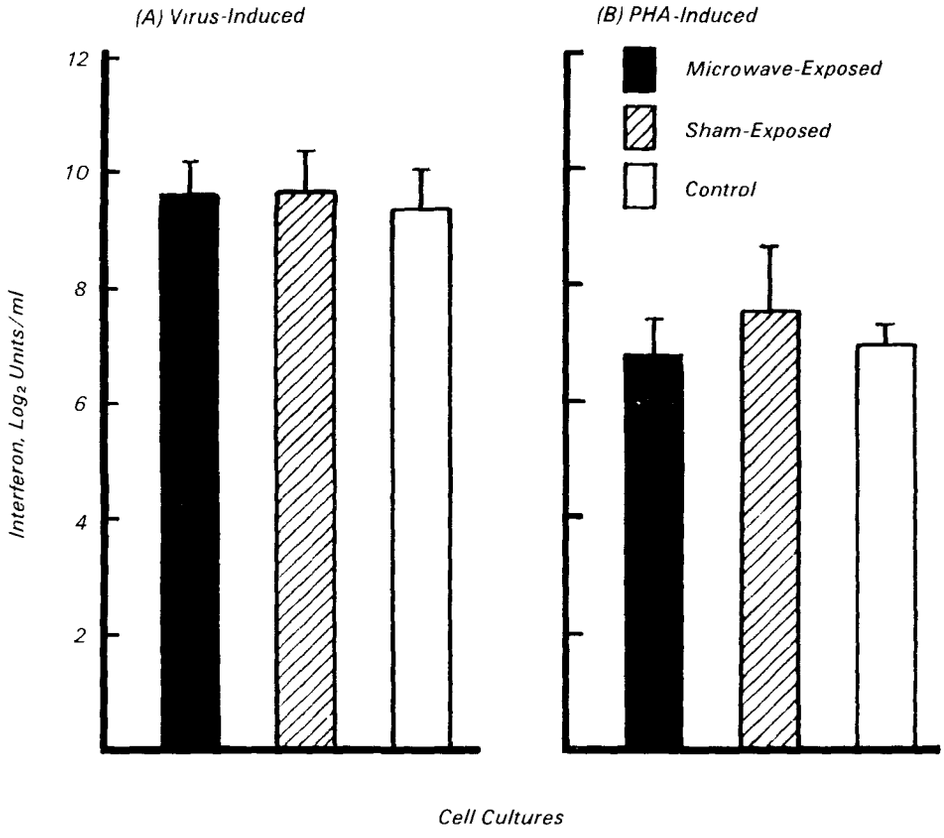

Figure 2. *Interferon-α and interferon-γ synthesis by microwave (mW)-exposed, sham-exposed, and unexposed (control) human mononuclear leukocytes. SAR = 4 mW/ml (A) Interferon-α titers present 24 hours after induction with influenza virus, and (B) Interferon-γ titers present 72 hours after induction with PHA are shown Columns indicate mean log_2 units/ml ± S.E*

these results are notable further for indicating that human leukocytes exposed to microwaves, as a potential physical stress factor, can respond normally to a second biological factor, such as the commonly encountered infectious agent, influenza virus.

The current studies do not exclude the existence of microwave-induced effects on human leukocytes resulting from exposures at greater SARs. Such exposures commonly produce effects that can be related to the degree and/or the rate of heating of the cell cultures or tissues *in vivo*. Furthermore, the current studies do not completely exclude potential microwave-induced effects resulting from exposure at similar SARs, but applied by almost innumerable different possible wave forms (frequencies, modulations, etc.).

Recommendations

The ubiquitous distribution of microwave energy and the potential differences between animal models and humans suggest that further investigations with human leukocytes and other cells may be warranted. The literature regarding microwaves includes animal studies reporting deleterious effects of exposure and animal studies reporting beneficial effects, over a broad range of SARs. Potential health hazards for humans should be further defined and limited, and potential health benefits, such as the use of microwave-induced hyperthermia in the treatment of cancer, should be further defined and expanded.

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The complete report, entitled "Microwaves, Hyperthermia, and Human Leukocyte Function," (Order No. PB 83-225 375; Cost: $7.00, subject to change) will be available only from:
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