MICROWAVE-INDUCED CATARACTS OF THE EYE LENS STRATEGIES FOR MODELLING AND... (U) UNIVERSITY OF WESTERN ONTARIO LONDON DEPT OF BIOCHEMISTRY J R TREWARTHAN JUN 03
MICROWAVE INDUCED CATARACTS OF THE EYE LENS; STRATEGIES FOR MODELLING AND PREVENTION IN VITRO AND IN VIVO ANNUAL SUMMARY REPORT JUNE 1983

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Microwave-Induced Cataracts of the Eye Lens: Strategies for Modelling and Prevention \textit{In Vitro} and \textit{In Vivo}

Annual Summary Report

DR. JOHN R. TREWITHICK

June 1983

June 1982 - May, 1983

Supported by

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Fort Detrick, Frederick, Maryland, 21701

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LONDON, ONTARIO, CANADA N6A 5C1

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**Microwave-Induced Cataracts of the Eye Lens:**

**Strategies for Modelling and Prevention In Vitro and In Vivo**

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**Rat eye lens, microwave irradiation, pulsed and continuous waves.**

**Tissue culture, medium 199, elevated temperature, globular degeneration, cataract vitamin E prevention.**

**We have developed a unique system for study of the development of microwave-induced cortical cataracts of the eye lens, using as a model system the rat lens incubated in circulating tissue culture medium at controlled temperature, while being exposed to microwave irradiation. Although in unirradiated controls no cataracts occur, we have used levels of microwave irradiation at which characteristic cataractous opacities with associated globular degeneration occur. We plan to continue our studies of microwaves, using the following**
techniques: (1) scanning and (2) transmission electron microscopy, (3) high
voltage electron microscopy, (a powerful technique for detecting alterations
in cytoskeleton structure), (3) cryological electron-stimulated detection
of x-rays (EDX) (a technique for examining the ionic composition of fresh
tissue), and (5) biochemistry. In these studies we hope to elucidate the
mechanism of the previously unreported unique effects of high energy pulsed
microwaves (noted below), which appear to be related to thermoacoustic trans-
duction. In the studies to date we have established several cataractogenic
doses but not looked at the minimum effective dose, and have noted several
features of the irradiated lens which are apparently related to the power,
temperature and duration of the irradiation and to the mode of delivery (con-
stant amplitude (CW) or high energy pulsed (Pu)). Important observations
in this system include the following: (1) apparent separation of effects
due to temperature from those arising from electromagnetic radiation (2)
effects such as large globules due to microwave irradiation at 37°C which
are only found at temperatures of greater than 45°C (3) tentatively a
suggestion of reciprocity between dose rate and time of exposure, which
requires further study (4) more damage caused by Pu than by CW microwaves
at the same power and time of exposure (5) apparently uniquely for Pu, holes
were detected in the cell membrane by SEM in samples fixed immediately after
exposure (6) capsular stretching and tearing (apparently caused by mechanical
forces) after exposure to Pu.
ABSTRACT

Rat ocular lenses were studied after fixation and critical point drying of the tissue by scanning electron microscopy (SEM) following exposures to elevated temperatures and/or microwave irradiation in a thermostatically controlled chamber. In this way, the temperature of the lens bathing medium was set independently of the temperature increase normally associated with application of microwave power. Irradiations were done at seven specific absorption rates (SAR) for three durations. These were accomplished at 918 MHz in WR975 waveguide with either pulsed (Pu) or continuous wave (CW) radiation of equal average power. The parameters of the (Pu) radiation were selected to maximize the production of thermoacoustic expansion.

In addition to the types of damage noted on previous progress reports: (1) immediately after 6 min. high energy pulsed microwave irradiation, at (a) SAR 10 and (b) 4 mW/g respectively: (a) loss of cortical fiber cell attachment to capsule coupled with distortion of fiber cell ends, and (b) disorganization of differentiating equatorial epithelial cells. (2) when ATP levels were determined for lenses irradiated as above, large dose-related increase in ATP concentrations in the epithelium and dose unrelated decreases in cortical ATP levels were observed immediately following 6 min irradiation.

SUMMARY

Over the four year period of this grant, the initial aims of this project were to develop techniques for incubating intact rat lenses in vitro in order to study the development of cataracts when lenses are exposed to elevated temperatures and/or microwave irradiation. We planned to (1) establish cataractogenic temperatures and SARs for irradiation in vitro and (2) investigate the mechanisms of cataractogenesis in such lenses. In a second stage, when suitable irradiation facilities existed, we planned to study the process of microwave cataractogenesis in vivo and to correlate the experimental results with those found previously for the in vitro work.

Initial studies, now published, indicated a linear correlation between depth of cataractous globular degeneration and temperature when the lens was exposed to a short period of temperature elevation and post incubated for a period of 48 hr. This unexpected linear relationship was found between 37°C and 50°C; in addition, at 47°C and 50°C some very large globules were formed. Surprisingly, apparently because they had been fixed by the high temperature lenses exposed to 60°C were clear and had lens cells of normal morphological appearance. D-a-tocopherol acetate when added to lenses before incubation at 41°C, prevented most of the globular degeneration observed at this temperature.
In initial attempts to expose the lenses to microwaves, a system was devised to circulate rapidly thermostatted coolant around the lens while it was being irradiated. This system permitted experimental separation of heating effects in the lens from effects due to electromagnetic radiation, since there was no measurable temperature elevation in the lens with respect to the surrounding medium even at highest microwave exposure levels tested. Irradiation was performed for two exposure times and at three SAR values.

The results of the irradiation indicated that the effect of the electromagnetic radiation itself could be considered to be equivalent to heating, since at the highest dose rate and 37°C, large globules were formed, which would otherwise have been reported at 47°C, equivalent to a temperature elevation of 10°C. Pulsed irradiation at high SAR values appeared to result in holes in the surface of cells, a novel observation which could indicate that thermoacoustic expansion is causing mechanical damage to cell membranes. Irradiations with pulsed radiation of 918 MHz were done at several different times and specific absorption rates (SAR) for several durations in order to explore a possible reciprocal relationship between dose rate and time, and to test whether any accumulative effect could be detected at low SAR values for periods up to 1 hour. Lenses were fixed immediately after exposure. The parameters of the pulses were varied to explore whether the biological effect seen was sensitive to a particular pulse parameter: duration, peak power, or power per pulse. The results are summarized as follows:

1. The extent of damage was measured by the depth of granular degeneration in the equatorial region in lenses fixed immediately after irradiation. Lens fiber cell effects were detected after 1 hr irradiation at the SAR values of 40 mW/g, 20 mW/g and 10 mW/g. When regimens delivering approximately the same total dose per unit weight (joules/kg) were tested, these depths were similar for similar total doses. When the area containing holes was included, they did not overlap the depths measured for two other total doses, each differing by a factor of approximately three fold from the previous.

2. At any particular SAR, the maximum depth of degeneration increases as a function of time of irradiation during the 1 hr period tested.

3. At any particular exposure duration, the maximum depth of degeneration increases as the SAR.

4. At low doses, with fixation immediately after irradiation, damage can be detected at dose rates as low as ½ W delivered to the incubation chamber containing the lens in 50 ml medium (SAR = 10 mW/g) after a 1 hr exposure.
FOREWORD

A. List of Professional Personnel Employed on This Project

   Principal Investigator - Dr. John R. Trevithick, Ph.D.
   Research Associate - Dr. P. Jill Stewart-DeHaan, Ph.D.
   Research Associate - Dr. Madhu Sanwal, Ph.D.

B. Animal Care

   In conducting the research described in this report, the investigator adhered to the "Guide" for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Science - National Research Council, U.S.A.
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INTRODUCTION

Since the collaborative development of an in vitro technique for experimentally distinguishing the cataractogenic effects of microwaves due to (1) elevated temperatures and (2) the electromagnetic field (Stewart-DeHaan et al., 1980) several generally accepted concepts regarding microwave cataractogenesis have been felt to deserve more detailed scrutiny. Such ideas as a temperature threshold for microwave cataractogenesis, which have been developed almost exclusively for CW microwaves, have ignored the possibilities for mechanical damage to the eye and its lenses as a result of the thermoacoustic expansion caused by high energy pulsed microwaves (Pu) similar to those commonly in use in military radars.

In the experiments reported here, we used the system we have devised for microwave irradiation of rat lenses in vitro. This permits the temperature of the lens bathing medium to be varied independently of the irradiating field, and examination of the lens by SEM following fixation (1) immediately after irradiation, or (2) following 48 hr incubation in M199. The changes are compared with known changes involved in the course of cataractogenesis induced by several toxic agents such as elevated glucose levels in M199, elevated temperature alone, or toxic drugs such as hygromycin B.

In these experiments we wished to study the questions: "Is there a reciprocal relationship between time and dose rate of microwaves (defined as Specific Absorption Rate, S.A.R.) required to induce a certain amount of cataractogenic damage in a lens?" We have explored as well the minimum dose and pulse parameters necessary to cause damage discernable in lenses fixed immediately after irradiation.

In our previous (1981) progress report we presented preliminary data outlining (1) several biochemical analytical techniques we have been developing to permit analysis of ATP, NADPH, NADH, vitamin E, O2 superoxide radical levels and lens protein alterations during the course of the cataractogenesis and (2) high voltage electron microscopy, which we hope to apply to single lens fiber cells from the equatorial area in which the initial changes in microwave cataracts occur.

MATERIALS AND METHODS

a) Microwave Exposure Apparatus

Rats (150-200 g) were sacrificed by decapitation and the lenses removed. Lenses with intact capsules were placed in M199 in culture tubes or in an apparatus in which they could be bathed in circulating phosphate buffered saline (PBS) at controlled temperatures while simultaneously being subjected to microwave radiation. Intact lenses were exposed to elevated temperatures for periods of up to one hour, followed by incubation in M199 at
35.5°C for two days. Some lenses were pre- and post-exposure incubated in M199 with 2.4 μM D-α-tocopherol (vitamin E) added in order to explore possible prophylactic effects for exposure to elevated temperatures.

Microwave radiation was delivered to the lens holder in two modes, Pu and CW. The lens holder and waveguide structure and the exposure system block diagram were illustrated in our 1981 progress report and in the attached paper, (Stewart-DeHaan et al, 1982). In the CW case, the signal was generated by a Hewlett Packard (HP) 8690B Sweep Oscillator with an HP 8699B plug-in, filtered by an HP 360B low pass filter to remove harmonics, amplified by an Amplifier Research 4W1000 amplifier and a Varian VZL-6943G1 TWT amplifier and then filtered again to remove harmonics and noise generated by the TWT. The power was adjusted to the desired level by setting the output level from the sweep oscillator. In the Pu case, the signal was generated by an Epsco PH40K and passed through a PAM-TECK UTA1017 isolator to protect the pulsed source from excessive reflected power. In either case, the signal was passed through a coax-to-waveguide adaptor (Dielectric Communications C-42061-501). A reflectometer (Dielectric Communications D-40588-502) was used in conjunction with HP 435A power meters to monitor forward and reflected powers. The impedance match to the lens holder was done using a triple stub tuner designed and built at Walter Reed. Typically, it was possible to adjust the tuner so that the reflected power was 15-18 dB below the forward power with the lens holder in place. The lens holder was located ½ guide wavelength from the waveguide shorting plate placing the lens holder in the maximum of the electric field. The vertical position of the lens holder was adjusted so that the lens was approximately at the center of the waveguide.

b) Microwave Exposure Conditions

Average transmitted powers were 60W, 20W, 6W, 2W, 1W, 0.5W, 0.2W and zero W for the shams. The corresponding SARs (as determined by measuring temperature elevation at the lens site with the coolant flow off) were 1.0-1.2 W/g, 300-400 mW/g, 100-120 mW/g, 40 mW/g, 20 mW/g, 10 mW/g, 4 mW/g and zero respectively. Initially, the bath offset temperature to meet the final temperatures was 1.4-1.6°C at 60 W of average power (with coolant flow), 0.3-0.4°C at 20 W and very close to zero at 6W and below. The bathing medium was phosphate buffered saline (PBS). Flow rate was 600 ml/minute. More recently, modifications to the circulating coolant system have permitted reductions in the offset temperature required to approximately 0.3°C at the highest SAR with proportional reductions at lower SARs by utilizing 1200 ml/min flow rates.

In the pulsed case, average power was set by variation of the pulse repetition rate. The peak power and pulse rise time for "standard conditions" were constant at about 24 kW and 0.1 to 0.5 sec, respectively. The pulse width used routinely was 10 - sec,
optimum for thermoacoustic expansion; in experiments testing peak power it was sometimes varied to 48 KW and pulse width varied from 5 μsec to 20 μsec at a maximum.

Lenses were fixed either immediately after irradiation or after the 2-day incubation in M199. After treatment with Karnovsky's fixative for 48 hours at 4°C the lenses were transferred to 0.1 M Na-cacodylate buffer, dehydrated in an alcohol series to acetone and critical point dried with CO₂. Each lens was quartered and attached in an SEM stub with silver daube paint, sputter-coated with gold-paladium and examined in a Hitachi HHS-2R SEM at an accelerating voltage of 20 KV.

c) High Voltage Electron Microscopy and Lens Fiber Cells Treated to Extract Soluble Cytoplasmic Components

These techniques were described in our progress report for 1981.

d) Cryological-SEM-EDX Sample Examination

Using matching funds ($150,000) from the University's Academic Development Fund, the purchase of the instrument package for this technique is underway. It is expected to be installed and operating in Autumn of 1983.

Fresh lenses are frozen on a cryostage, using octanol to attach them to the cryostage, and freeze-fractured. The frozen fractured specimen is transferred on a cold stage cooled with liquid nitrogen to the SEM specimen chamber and examined. A suitable area is chosen for x-ray analysis and the spectrum recorded. Two dimensional analysis of three elements simultaneously can be performed, giving a picture which corresponds to the SEM picture.

e) Hi-chemistry Sample Preparation and Staining

This was described in 1981.

f) Water-Soluble Embedding method for Light Microscopy

This was described in 1981.

g) Biochemical Techniques

Techniques for the following were described in 1981:

Protein, adenylate nucleotides, NADPH, NADH, superoxide anion, glutathione, vitamin E and vitamin C.

h) Modulation Transfer Function

Progress towards this measurement was reported in 1982. In the current year, no experiments are reported.
NEW RESULTS

As described in the attached paper and appendix with Figure 1 we found mostly equatorial damage, which when compared to the results reported in 1982 was more with pulsed than CW at the same dose. There was an approximately reciprocal relationship between time of exposure and the dose rate required to produce a particular depth of degeneration (summarized in manuscript). Globules, granular cell surfaces or holes occurred to similar depths of damage for similar total dose.

High resolution light microscopy is a new technique. The attached manuscript figure 6 illustrates typical morphological changes in such lenses by this new version of a conventional technique. Figure 1 illustrates such microscopy of changes observed in lenses fixed immediately after irradiation at low doses of pulsed microwaves, but at elevated peak power; such high peak powers would be found in the vicinity of military airport and ship-borne radar installations.

At the second lowest dose rate attempted following 1 hr at 0.5 W (4 x EP) lenses showed abnormal morphology at the immediate postequatorial connections of fiber cells to the capsule. The cell ends appeared twisted and deformed, whereas shams exposed to similar conditions were not adversely affected. After irradiation at the lowest dose rate (0.2 W, 4 x EP), abnormal arrangement of the differentiating equatorial epithelial cells was observed.

Table 1 after Fig. 2 in the appendix, represents a more detailed analysis of changes in the level of ATP, in lenses subjected to low dosages of microwave irradiation. In two separate sets of experiments, (Table 1), (Fig. 2) we compared the ATP levels per mg protein in fresh rat lenses, and those sham-irradiated or irradiated for 6 minutes. As can be seen, the levels of ATP per mg protein in capsule plus epithelium (CE) is slightly less than cortex and much larger than the level in the nucleus. When irradiated, the level of ATP in CE and cortex appears to decrease to about half that of a fresh lens. Alteration of the modulation conditions to give the same average power with double the peak power resulted in a striking increase in epithelial ATP levels, up to almost that of normal fresh lenses. Further alteration of modulation conditions at the same average power to give four times the energy (Pu, 4 x EP) per pulse (as compared to fresh controls) resulted in a striking increase in epithelial ATP per mg protein up to approximately three times the fresh epithelium while maintaining similar cortical ATP levels to that found at 0.5 W under standard conditions. Comparison of these conditions to CW indicated that the increase in epithelial ATP levels and decrease in cortical ATP's at 65W SAR (% W/g) at 37°C, were roughly equivalent to the elevation in epithelial and cortex caused by the 0.5 W, 4 x EP condition.
DISCUSSION

In considering the possible hazards of microwave irradiation for military personnel, it was appropriate to consider not only the effects of acute damage which might develop following inadvertent exposure to high doses under combat conditions, but also possible long-term effects which might ensue following low level exposures over a longer period of time. Although such experiments could be conducted in a living animal, it was deemed advisable, in beginning these experiments, to use a more sensitive system in which the cataractogenic response occurs rapidly and to use large doses which would ensure that a measurable effect was found. Once threshold studies (reported here) were conducted to determine the minimum level of microwaves resulting in detectable irradiation an appropriate level to check was established.

The in vitro system developed for exposing lenses in medium to the stress of chemical compounds, temperature, and irradiation permits the precise manipulation of these factors, whereas, in vivo, only an approximation is possible. In addition, the various types of damage and their degree or extent are more readily and rapidly detectable and assessable; for example, incubation with 55.6 mM glucose in M199 results, within 2 days, in development of both globular degeneration and opacity, while the same process in the intact diabetic animal would require as much as 6-8 weeks to reach a similar degree of degeneration.

Taken with the attached paper our new results indicate most damage at the highest power per pulse at high peak power (48 KW). They extend detection of damage from previously reported (1982) dose rates of 1 and 2 watts (approximate SAR 10 and 20 mW/g, respectively), now to include several lower dose rates at which damage was previously reported using standard pulse conditions (10 μsec peak power 24KW). The lowest dose rates studied, correspond to 0.5 W (SAR 10 mW/g approximately) and 0.2 W (SAR 4 mW/g). The type of damage seen at 1 and 2 W in immediately fixed lens samples, consisted of some foam and globular degeneration and rather large areas of cells with granular cell surfaces. The lack of fiber cell adhesion to the capsule and distortion of the fiber cell ends in the post equatorial capsular region seen at 0.5 W and the disorganization of epithelial cells (ep) at 0.2 W are consistent with lesser degrees of membrane or cell surface damage. The holes seen at higher dose rates, in the paper, appear to be a later stage in a progressive series of events in membrane damage. Such membrane damage appears to be related to physical damage due to pressure waves occurring as a result of the thermoacoustic expansion caused by pulsed microwaves (Foster and Finch, 1974), since these effects are found to be more pronounced when at the same average power, the microwaves are delivered in pulsed mode rather than as continuous wave. The theoretical basis for such physical damage has been discussed by Lin (1978); he ascribes the sound waves to thermoelastic expansion. Some uncertainty remains regarding the correspondence between his
theoretical treatment of thermoelastic stress and the measured
differences in experimental damage to the irradiated rat lenses,
which we have found. A particularly vexing question is why
increasing the pulse peak power or the power per pulse appears to
cause significantly more damage even though the average power and
total dose are held constant. As Lin admits (page 157) "The sound
pressure generated in a surface-stress-free spherical head model
is, therefore, smaller than known results." (Apparently by a
factor of about 6.5). This discrepancy, which he addresses by
modifying the model, indicates the need for further development
of theoretical treatments. The large difference in response of
ATP levels in the epithelium and cortex (see below) seen when
pulsed and CW modes are compared (see below) is consistent with
an explanation whereby thermoacoustic pressure waves lead to
membrane damage and epithelial cell ATP production increases
coincident with cortical ATP decreases. This presumably occurs
because of mitochondria, the main cell organelles producing ATP,
being present in epithelial cells but absent from cortical cells.
Some pressure wave-induced change in conformation or spatial
interaction of mitochondrial proteins could presumably lead to
enhanced ATP production. Alternatively, the rate of hydrogen ion
transport through ATP-generating channels could be enhanced by
the pulses of increased pressure. In this connection, an
investigation of ultrasound-induced pressure waves on ATP
production might be useful for the purpose of comparison.

The development of a technique for measurement of modulation
transfer function of fixed rat lenses was reported last year. No
further work has been undertaken this year, since the
morphological and biochemical experiments appeared to be of
higher priority, and funds were limited.

Special notice should be taken of the data reported in the
paper, supporting an apparent proportional increase in depth of
globular degeneration, as a function of dose rate, for pulsed
irradiation and the similar extent of damage observed for
different SARs at times which resulted in a similar total dose
being delivered to the sample. The possibility of reciprocity,
generally recognized as a feature of ionizing (but not of
nonionizing) irradiation, has not been suggested to date for
microwave irradiation; these data support the idea that such a
relationship is approximately true for the short times we used.
If also true in vivo, such reciprocity could have important
implications for long term chronic exposure to low levels of
pulsed microwave irradiation. This would imply that long term
exposure to a low level of pulsed microwave irradiation, such as
might occur in military personnel working in the vicinity of a
radar installation, might result in increased risk of
cataractogenesis. For examples, in vitro even though little
damage can be seen after 2W exposure for 6 min, more extensive
damage is noted after exposure to 2W pulsed energy for 20 min
(see paper). This makes it seem very likely that if exposures at
low dose rates are conducted for a long enough period of time (several hr), the damage would be additive. This hypothesis should definitely be tested.

The mechanism of cataractogenesis in the isolated lenses following exposure to microwave irradiation is still not well-defined. Nevertheless, certain features of this process were suggested to the experiments on high voltage electron microscopy reported in our 1981 progress report. The peculiarly similar appearance of the cytoskeleton in microwave-irradiated fiber cells, and tissue culture cells treated with cytochalasin D, is consistent with the idea we have presented previously, that in cortical cataracts the critical change leading to globular degeneration and opacity is a change in the cell cytoskeleton (Mousa, Creighton and Trevithick, 1979).

The decreased cortical ATP levels reported here for microwave irradiated lenses are consistent with a mechanism for globular degeneration related to actin super contraction as a result of decreased ATP - a sort of "cellular level rigor mortis". This, however, is only one of several possible mechanisms which are listed below.

The biochemical results on cataractogenesis in vitro induced by heat, microwaves and glucose are consistent with several possible mechanisms for globular degeneration:

1. The lowering of cortical ATP levels would be expected to result in supercontraction of the actin microfilaments.
2. The increased level of superoxide radicals with time would be expected to result in oxidative damage to sulfhydryl groups, or oxidation of unsaturated fatty acids, and proteins of membranes, as might the previously reported (by Kinoshita's group) decreased or of reduced pyridine nucleotide or glutathione, reported for radiation cataract recently by Reddy's group (Giblin 1980) level of vitamin C.
3. A decrease of potassium/sodium ion ratio and increase in calcium, which might result from cell membranes with leaks or holes, would be expected to cause a change in contraction of cytoplasmic microfilaments, since potassium and calcium are required for the process of micofilament contraction.
4. The possibility of some prostaglandin, or thromboxane, or prostacyclin metabolite being generated to a larger extent acting on a cell membrane or cytoskeletal receptor; this of course would be reduced by biological antioxidants such as BHT, glutathione and vitamin E.

Experimental design in the future is expected to be directed towards analyses which will enable us to determine whether these mechanisms (singly or in combination) actually function, once appropriate conditions of irradiation are selected.
ACKNOWLEDGEMENTS

We wish to acknowledge the excellent technical assistance of Mr. Leon S. Butler and Ms. Andrea Hanington for performing the surgical procedures to remove the lenses. We thank Mr. Warren Foster for his contribution in designing and building the glass lens holders. Grants from the U.S. Army Medical Research and Development Command (DAMD-78-G-9449), the MRC of Canada, National Eye Institute (U.S.A.) (EY1927) and the E.A. Baker Foundation of the CNIB are acknowledged with thanks.
LITERATURE CITED


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Table 1  ATP levels in lens areas exposed to microwave irradiation. Lenses were exposed to microwaves as described in Methods and in Figures 1 and 2
TABLE 1: ATP Levels in Areas of Lenses Exposed to Microwave Irradiation

Experiment Series 1

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Fresh</th>
<th>$\frac{1}{2}$ W Pulsed (ng ATP/µg protein)</th>
<th>$\frac{1}{2}$ W. 2 x Pk. P.</th>
<th>$\frac{1}{2}$ W. 4 x E/Pu</th>
<th>65 W. CW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>9.32</td>
<td>3.16 ± 0.75</td>
<td>8.71 ± 8.05</td>
<td>24.3 ± 4.60</td>
<td>25.8 ± 5.94</td>
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<tr>
<td>Cortex</td>
<td>11.7</td>
<td>6.10 ± 0.21</td>
<td>2.62 ± 3.08</td>
<td>5.63 ± 1.61</td>
<td>3.57 ± 2.42</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0.299</td>
<td>0.264 ± 0.083</td>
<td>0.205 ± 0.066</td>
<td>0.524 ± 0.249</td>
<td>0.604 ± 0.389</td>
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</tbody>
</table>

Experiment 2

<table>
<thead>
<tr>
<th>Fresh</th>
<th>$\frac{1}{2}$ W Pulsed (ng ATP/µg protein)</th>
<th>$\frac{1}{2}$ W. 2 x Pk. P.</th>
<th>$\frac{1}{2}$ W. 4 x E/Pu</th>
<th>Sham 6 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=4</td>
<td>n=4</td>
<td>n=3</td>
<td>n=3</td>
</tr>
<tr>
<td>Epithelium</td>
<td>3.96 ± 2.98</td>
<td>1.34 ± 0.271</td>
<td>1.72 ± 1.18</td>
<td>4.69 ± 3.95</td>
</tr>
<tr>
<td>Cortex</td>
<td>7.86 ± 2.02</td>
<td>4.33 ± 2.34</td>
<td>6.61 ± 2.73</td>
<td>6.26 ± 3.54</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0.430 ± 0.313</td>
<td>0.272 ± 0.0908</td>
<td>0.193 ± 0.0230</td>
<td>0.384 ± 0.140</td>
</tr>
</tbody>
</table>

Experiment Series 2

<table>
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<tr>
<th></th>
<th>Sham 60 min</th>
<th>0.2 W 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=3</td>
<td>n=3</td>
</tr>
<tr>
<td>Epithelium</td>
<td>0.628 ± 0.238</td>
<td>0.125 ± 0.167</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.646 ± 0.273</td>
<td>0.591 ± 0.170</td>
</tr>
<tr>
<td>Nucleus</td>
<td>0.0528 ± 0.0263</td>
<td>0.0812 ± 0.112</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

Figure 1. Thick sections of plastic embedded rat lenses: (A) Control sham irradiated showing equatorial region from lens as compared to the same area from irradiated lenses (B&C) exposed to 0.2 W, 60 minutes pulsed (48 KW, 20 μsec, 4 EXP) microwave irradiation at 37°C and then fixed immediately. Note disorganization of cell arrangement in equatorial zone (eq) outer fibers and epithelial cells (ep), as compared to sham-incubated control.

Figure 2. ATP levels in different zones of irradiated or sham irradiated lenses dissected into capsule plus Epithelium (E), cortex (C) and nucleus prior to homogenization in guanidinium chloride followed by ATP determination using the luciferin-luciferase assay. The width of the bar graph designates the protein concentration, while the height of the bar designates ATP specific activity in mg/μg protein.
ATP CONTENT OF IRRADIATED RAT LENS FRACTIONS

ATP CONCENTRATION (ng/μg PROTEIN)

PROTEIN (mg)

EPITHELIUM

CORTEX

NUCLEUS

FRESH

65 W. CW

½ W. Pu

½ W. Pu2xPkP

½ W. Pu4xE/Pu
END

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