Examination of the Cornea Following Exposure to Microwave Radiation

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This study was designed to detect alterations in the corneas of rabbits caused by multiple exposure to either 2450 MHz continuous wave (CW) or 2860 MHz pulsed (P) radiation at an average power field density of 225 mW/cm². Hematoxylin and eosin stained sections of corneas were examined. In some cases, the pattern of tritiated thymidine (TH^3) uptake into corneal cells was evaluated by autoradiography. Radiation did not appear to influence the normal cornea or the healing process in the wounded cornea.

DURING THE COURSE of studying the cataractogenic potential of 2450 MHz continuous wave (CW) radiation, several investigators have noted the development of corneal lesions in some of their animals (1,3,4). According to these reports, corneal effects—variously described as clouding, ulceration or burns—can occur at field power densities as low as 200-300 mW/cm² (3). The histopathology of these lesions has not been reported.

The purpose of this study was to examine histologic sections of corneas from rabbits irradiated with either 2450 MHz continuous wave (CW) or 2860 MHz pulsed radiation, at field power density levels high enough to cause extraocular effects such as conjunctival injection and chemosis, yet too low to produce corneal lesions detectable by slit-lamp biomicroscopy. In our laboratory, field power densities averaging 225 mW/cm² satisfied these criteria. In addition, the corneas of some animals were wounded and, with the aid of autoradiography, the course of healing under the influence of daily irradiation was followed. This technique has been used on several occasions to uncover the subtle effects of other agents on the cornea (6, 7, 8).

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The animals used in this study were handled in accordance with the provisions of Public Law 89-544 as amended by Public Law 91-579, the 'Animal Welfare Act of 1970' and the principles outlined in the 'Guide for the Care and Use of Laboratory Animals,' U. S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

MATERIALS AND METHODS

Hardware: Continuous wave (CW) radiation was produced by a Rhode and Schwarz UHF power signal generator, Type SCRD, Model BN41004, driving a Manson Laboratories TWT power amplifier Model 730. Frequency was monitored off the Rhode and Schwarz with a Narda Frequency Meter, Model 802B.

Pulsed radiation at 2860 MHz was provided by a Raytheon Model 4J31 magnetron driven by a Manson Laboratories Pulse-Tube Modulator, Model 275. For all pulsed exposures, the pulse repetition frequency (PRF) was 500 Hz and the pulse width was 1 μs. Frequency modulation parameters were monitored with a Polarad Model SA-84WA spectrum analyzer.

Both CW and pulsed radiations were transmitted via S-band waveguide to a standard gain horn oriented to give a vertically polarized electric field. Forward power was monitored with a 30 db waveguide directional coupler and a Hewlett-Packard power meter, Model 432.

The horn radiated into a chamber 2.25 m × 2 m × 3.6 m constructed with copper screen and lined with microwave-absorbing material.

Animals: Adult male New Zealand white rabbits weighing between 2-3.6 kg were used throughout the study. Prior to use, each animal was examined carefully by slit-lamp biomicroscopy to exclude animals with corneal abnormalities.

Placement in the Field: Unanaesthetized animals were placed in a plexiglass restrainer and positioned in the irradiation chamber so that the cornea to be irradiated was tangent to a plane of the desired power density. The rabbits were checked frequently during irradiation to insure that they remained properly positioned with their eyes open.

Dosimetry: Measurements were made for CW at 2450 MHz with a Narda probe, Model 8100. Pulsed radiation at 2860 MHz was measured with a Ramcor Model 1200 densitometer.

The rabbit's head was positioned about 75 cm from the horn. Measured with the Narda probe, the field density at this distance was uniform within 0.5 db over a cross-sectional area of 600 cm² and diminished in intensity as approximately $1/r^2$, a characteristic of the

far field.

Plexiglass can produce distortion of the microwave field (2). To minimize the uncertainty in the power field densities listed in Table I, measurements were made with the Narda probe placed in the restrainer in the position normally occupied by the rabbit's head. The field densities were increased approximately 40% above those measured in the absence of the plexiglass.

Corneal Wounding: The rabbits were anaesthetized with intravenous Surital (sodium thiamylal 25 mg/kg) and Ophthaine (proparacaine hydrochloride) was instilled over the cornea. The cornea was wounded using a serrated circular attachment to a high speed drill. A 0.5 × 10 mm × 0.2 mm deep lesion was produced perpendicular to the palpebral fissure. The cornea was protected from drying during the procedure and the recovery from anaesthesia.

Autoradiography: In the autoradiographic technique, tritiated thymidine is presented to cells for a short period and the cells are then mounted on a slide and fixed. A layer of photographic emulsion is then deposited over the preparation. The low energy beta radiation will affect the silver halide in the emulsion over those structures which have incorporated the radioactive isotope. Black grains appearing over the nucleus of the cell when the emulsion is developed, shows that tritiated thymidine was incorporated into deoxyribonucleic acid (DNA) during cellular division. In a tissue slice, the distribution of black grains indicates the degree and location of cellular division.

The rabbits were anaesthetized with intravenous Surital, and Ophthaine was instilled over the cornea. Five microcuries of tritiated thymidine were injected into the anterior chamber through a 30-gauge needle introduced at the peripheral edge of the cornea. The eye was gently massaged to promote mixing, and the needle was then carefully removed to minimize leakage of aqueous humor containing the label.

Two hours later, the rabbits were sacrificed by rapid intravenous injection of Somlethol. To facilitate handling

TABLE I. IRRADIATION PROTOCOL.

Group	Sub Group	Corneas for Histo/ Autorad°	Freq/ Mode	Field Density# (mW/cm²)	Exposure Duration (min)	Number Consecutive Exposures	Exposure Interval (Days)	Hours/Days Sacrificed after Wounding	Hours Sacrificed after Last Exp.
I	Unirradiated, unwounded controls								
	1	12/12							
II	Irradiated wounded								
	2	4/0	2450/cw	225	30	1	—	—	0
	3	4/0	2450/cw	225	30	1	—	—	2
	4	4/0	2450/cw	225	30	4	1	—	2
	5	4/4	2450/cw	225	30	8	1	—	2
	6	4/0	2860/p	225	30	4	1	—	2
	7	4/4	2860/p	225	30	8	1	—	2
	8	4/0	2450/cw	330	10	1	1	—	2
	9	4/4	2450/cw	330	10	3	1	—	2
	10*	4/4	2450/cw	225	30	5(5/7)*	1,3	—	2
III	Unirradiated, wounded controls								
	11	4/4	—	—	—	—	—	6hrs.	—
	12	4/4	—	—	—	—	—	12hrs.	—
	13	4/4	—	—	—	—	—	1d	—
	14	4/4	—	—	—	—	—	2d	—
	15	4/4	—	—	—	—	—	3d	—
	16	4/4	—	—	—	—	—	4d	—
	17	4/4	—	—	—	—	—	8d	—
IV	Irradiated, wounded								
	18	2/0	2450/cw	225	20	1	—	6hrs.	2
	19	4/1	2450/cw	225	20	1	—	12hrs.	2
	20	7/3	2450/cw	225	20	2	1	1d	2
	21	4/0	2450/cw	225	20	3	1	2d	2
	22	2/0	2450/cw	225	20	4	1	3d	2
	23	6/2	2450/cw	225	20	5	1	4d	2
	24	2/0	2450/cw	225	20	9	1	8d	2
V	Irradiated, delayed wounding +								
	25	2/0	2450/cw	225	30	+	+	12hrs.	—
	26	2/0	2450/cw	225	30	+	+	1d	—
	27	2/0	2450/cw	225	30	+	+	2d	—

Field measurements have an estimated uncertainty of 10%.

* Subgroup 10 was irradiated 5 days a week for 5 weeks.

+ Group V was irradiated 5 days a week for 4 weeks, then wounded.

°The first number is the number of corneas examined histologically. The second number is the number of corneas that were also examined autoradiographically.

of the tissue and identification of the wound, Carnoy's fixative was immediately dripped over the corneal surface of the intact eye, and a small amount of aqueous was aspirated from the anterior chamber and replaced with fixative.

Corneas were excised and placed in Carnoy's fixative for 1.5 hours. After dehydrating in alcohol, the tissue was embedded in paraffin, sectioned to a 5-micron thickness, and mounted on glass slides. Those sections intended for histologic examination alone were stained with hematoxylin and eosin (H&E) at this point. Those sections for autoradiography were placed in xylene to remove the paraffin and taken through graded concentrations of alcohol in water to pure water. In the darkroom, each slide was dipped in Kodak NTB liquid emulsion, dried for 20 minutes at room temperature, and then stored in the dark for 10-14 days. The slides were developed by serially placing them in Kodak D19 for 5 minutes, short stop for 15 seconds, then acid fix for 3-5 minutes. The slides were rinsed thoroughly with water and stained with H&E. Each cornea yielded about 50-70 sections.

Protocol (Table I)

Group I consisted of unwounded control animals. Sections for autoradiography were prepared from each cornea.

Group II animals were irradiated according to the schedule in Table I. Individual subgroups (5,7,9, 10) were selected for study by autoradiography.

Group III was the control group for experiments involving wounding. All corneas were processed for autoradiography.

Group IV animals were wounded in the corneas, which were then irradiated daily while healing. The sampling interval is shown in Table I. Subgroups 19, 20, 22 included the only specimens available for autoradiography.

Group V animals were irradiated for 4 weeks (5 days on, 2 days off) prior to wounding the cornea in order to determine whether semichronic exposure to microwave radiation altered the pattern of healing.

In those studies involving wound healing, the corneas were wounded 2 hours prior to the first irradiation. This allowed the rabbits ample time to recover from the anaesthesia.

RESULTS

Although particular attention was focused upon the corneal epithelium in these studies, all portions of the cornea were evaluated.

Group I consisted of 12 corneas from 6 control animals. All corneas were processed for autoradiography to establish the basal pattern of DNA synthesis. For these corneas, one in eighty (range 1/70 - 1/90) cells had grain counts of over 3, the criterion for deciding that the cell was incorporating the label. The range

TABLE II. FEATURES OF WOUND HEALING OBSERVED IN CORNEAS OF CONTROL ANIMALS.

	HISTOLOGIC	AUTORADIOGRAPHIC
Before Wounding		
	Normal epithelium 3-4 cells thick Stroma homogenous appearing, only a few cells (keratocytes) seen between lamellae	About one in eighty basal epithelial cells incorporating label* No labelling of keratocytes
After Wounding		
0-12 Hours	Little cellular activity at wound site	Within 2 mm of wound margin, a marked reduction in basal epithelial cells taking up the label
12-18 Hours	Epithelium at wound margin thinned out as epithelial cells migrate into wound Polymorphonuclear keratocytes appear in stroma subjacent to the wound	
24 Hours	Wound floor lined with single layer of epithelial cells	An increased number of basal epithelial cells incorporating label surrounds the wound, 1 mm from the edge Some keratocytes around wound labelled
48 Hours	Wound floor 2-3 cells deep Polymorphonuclear keratocytes and mononuclear cells numerous in stroma	
4 Days	Epithelium on floor of wound 6-7 cells deep Epithelium at wound margin restored to normal thickness	On the floor of the wound, more than usual number of basal epithelial cells taking up label Peripheral to wound margin, activity decreasing toward normal

* tritiated thymidine (TH^3)

was similar when the two corneas of the same animal were compared.

In Group II, neither a) up to 8 consecutive days of 225 mw/cm² for 30 minutes either CW or pulsed, nor b) a total of 5 weeks (5 days on, 2 days off) of 225 mw/cm² CW for 30 minutes nor c) 3 days of 330 mw/cm² CW for 10 minutes had noticeable effect on the cornea as a whole, or on the number or pattern of cells undergoing DNA synthesis.

In Groups III-V the cornea was wounded. It was anticipated that slight alterations in any of the parameters mediating the healing response would be amplified by this means. No difference between irradiated and control groups was evident when the healing process was accompanied by daily exposure to 200 mw/cm² CW for 30 minutes, nor was there an effect on wound healing when wounding was delayed until after the cornea had been irradiated for 4 weeks (5 days on, 2 days off).

The features of wound healing observed (Table II) are in substantial agreement with those described by Hanna (5).

DISCUSSION

Naval radar systems operate in the range between 200-10,000 MHz. Our data indicate that 2450 MHz CW and 2860 MHz pulsed microwave energy does not affect the cornea at doses which far exceed those likely to be received by personnel under conditions of accidental exposure. We emphasize that our observations were made on the corneal effects of 2450 MHz and 2860 MHz radiation. Because of the relationship of the depth of penetration of the energy into the tissue to the frequency of the radiation, higher frequencies can be expected to be more efficient in producing thermal damage in the corneal epithelium. For example, at 2450 MHz 40% of the energy incident on the cornea is absorbed by the tissue; the remaining energy is reflected. At this frequency, the depth of penetration, defined as the distance at which the incident power density is reduced by a factor of 1/e, is approximately 1 cm (9). For an incident power density of 200 mW/cm², an effective corneal area of 1 cm², and a corneal thickness of 0.05 cm, approximately 4 milliwatts of power is absorbed by the cornea. For comparison, at 10,000 MHz, where the depth of penetration is approximately 0.05 cm, 50 milliwatts of power would be absorbed by the cornea. Clearly, further investigation into the possible injurious effects of radiation at higher frequencies is warranted.

CONCLUSION

Microwave irradiation of rabbits with 2450 MHz continuous wave (CW) or 2860 MHz pulsed (P) at an average power field density of 225 mW/cm² given daily for 20-30 minutes for up to 5 weeks had no detectible effect either on the normal or the wounded cornea. The pattern of tritiated thymidine uptake in both the normal and wounded cornea was unaltered by the radiation.

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