TERATOLOGICAL STUDIES OF FETAL EXPOSURE OF MICE TO RF RADIATION

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Abstract

The teratogenic potential (in utero damage of fetuses) of repeated whole body exposure of pregnant mice to low-level 148 MHz radiation is studied. The dames were exposed daily for one hour at 1 mW/cm^2 from day one to day nineteen of gestation. Preliminary results suggest significant fetal weight differences but no significant incidence of abnormalties and growth retardation.

Introduction

During the last decade the biological effects of nonionizing radiation have been extensively studied by many investigators. A few results indicated that the mammalian embryo is uniquely sensitive to the deleterious thermal effects of both microwave and radio frequency (RF) radiation.[1,2] With the proliferation of RF sources operating in the environment[3] there is an ever increasing need for information regarding specific biomedical effects of RF radiation. This research represents a study of the teratogenic potential (in utero damage of fetuses) of low-level 148 MHz repeated whole body irradiations on pregnant mice.

Methods and Materials

For the first experiment of this study 108 female and 27 male C_3H mice were derived within our own laboratory facilities. For the first replication 64 females and 16 males were derived. They were maintained under a natural light/dark cycle with ambient room temperatures of $25 \pm 2°C$ and relative humidity at $40 \pm 10\%$. When in their cages, the mice were allowed ad libitum access to lab chow and water.

Timed mating began when the animals were eight weeks of age. At 1800 hours of each day one male was placed in each breeding cage with four females. The breeders were segregated during 0700 and 0900 hours and examined for evidence of sperm plugs. Observation of a sperm plug confirmed copulation, and this day was marked day one of the gestational period. The female was then removed for irradiation and weighed daily throughout the experimental period. If copulation was not confirmed, the females were returned to the breeding cage for mating until a sperm plug was detected.

Irradiation Procedures

Plane wave fields at 148 MHz were generated in two rectangular coaxial exposure systems. The electric field was uniform within a horizontal region of 30 cm × 30 cm around the center of the chambers. Four positions within each ventilated chamber were selected for placement of the experimental animals. During exposure the animals were restrained by confining them in 2 stacked styrofoam cups. The cups are Dart Co. 8S12 squat cups with dimensions 8.89 cm top, 6.83 cm side and 6.83 cm base. The lids of the cups are plastic with ten 0.24 cm holes for ventilation. Although these dimensions allow animal movement with minimal stress, the mice tend to remain still during the restraining period. Also this confining method produced minimal distortion of the incident field.

The pregnant female mice were irradiated daily for 1 hour, 4 per chamber from day 1 to day 19 of gestation. The incident power density was 1 mW/cm^2, corresponding to 0.05 mW/gm of peak rate of energy absorption. As sperm plugs were detected in 80 mice during the 5 day mating period, 2 equal groups of 40 were formed to serve as shams and irradiates. Shams were mock-irradiated in identical nonenergized exposure systems. In the second experiment, 2 equal groups of 16 were formed as shams and irradiates.

At the 19th day of gestation, each pregnant female was euthanized with CO_2 gas, and the uterus was immediately removed via Caesarian section. Photographs were taken to document the position and number of implantations, stillborn, and resorbed fetuses. This procedural format was performed on 12 irradiates and 14 sham-irradiates or controls of the first experiment and 9 irradiates and 6 controls of the second experiment. Although the average gestation for nonexperimental mice is 20 days, 20 females of experiment I, equally divided control and irradiates, went to term at 19 days with natural births. In experiment II, 3 irradiates and 3 controls were permitted to complete natural term births. Weights of all natural birth fetuses were recorded. The growth pattern of these litters allowed to develop to adulthood (60 days) was monitored.

Results

For both experiments, of the 112 animals detected for copulation by presence of a sperm plug, 45 did not conceive. The results of those females that did conceive and whose fetuses were removed by Caesarian section are shown in Table 1. A total of 377 implantations were observed in 41 experimental animals. It is interesting to note that in irradiated animals a higher percentage of implantations occurs in the right uterine horn.

In experiment I, the percentage of fully resorbed fetuses and occurrence of abnormalities were slightly higher in the exposed group but the number of control stillborn fetuses were larger than in the exposed group. In experiment II, the distribution of the tabulated parameters were without difference.

A comparison of control and exposure mean values of several parameters was analyzed using the t-test. All variables were assumed to be independent samples, normally distributed, from populations that may or may now have equal variances. Therefore t was estimated with modified degrees of freedom via computer analysis. The significance level of the test (p value) was preset at 0.05. The statistical results are presented in Table 2. Both the Caesarian and natural birth fetal weights differed significantly with $p < 0.05$. There was no difference ($p > 0.05$) in the adult weights at 60 days of age for the natural born in experiment I.

The laboratory conditions and procedural format of both experiments were equivalent in all aspects. Therefore, the data was combined for Caesarian fetal

TABLE 1

Experimental Group	Number in Group	Total Fetal Implantations (ave. per animal)	Fetal Implantations in Uterine Horns		Normal Living Fetuses[a]	Stillborn Fetuses[b]	Fully Resorbed Fetuses	Abnormalities
			Right	Left				
Experiment I								
Control	14	126(9.0)	74	52	118	8(6.3%)	2(1/6%)	0
Exposed	12	120(10.0)	65	55	109	5(4.2%)	3(2.5%)	2(1.7%)[c]
TOTAL	26	246	139	107	227	13	5	2
Experiment II								
Control	6	49(8.2)	23	26	47	2(0.04%)	1(0.01%)	0
Exposed	9	82(9.1)	44	38	79	3(0.04%)	1(0.01%)	1(0.01%)
TOTAL	15	131	67	64	126	5	2	1

a Living as an indicator of fetal movement during delivery and not ability to sustain life afterwards.

b Includes partially resorbed fetuses and those with abnormalities

c Evidence of cephalic hemorrhage. Other abnormalities could have included micrencephaly, exencephaly, cleft palate, omphalocele, and club foot.

d Evidence of cleft palate.

TABLE 2

Parameter	Experiment	
	I	II
Caesarian Fetal Weight	+	+
Natural Birth Fetal Weight	++	+
Adult Male Body Weight	–	a
Adult Female Body Weight	–	a
Caesarian Fetal Weight for Combined Experiments		–

– Not Significant (p > 0.05)

+ Significant (0.01 < p < 0.05)

++ Significant (p < 0.01)

a Not determined

weight and statistical analysis was performed. As shown in Table 2, with p > 0.05, the mean weights were not different.

Discussion

The specificity of any teratogenic agent is related to the stage of gestation at which the teratogen is administered. Although the fetuses were irradiated through all 5 developmental periods of gestation, the authors believe that preimplantation and early organogenesis stages would be the most sensitive, resulting in fetal resorption or pronounced intrauterine growth retardation, respectively. Yet, the effects of irradiation at later periods of development could result in the largest degree of permanent growth retardation; thus, a more sensitive index of biological damage than is teratogenesis.

To substantiate identical repetitions and to search out which groups are different from each other, care must be taken to select an additional test which will not increase the overall type 1 error. Between group analysis for control and exposed groups has not yet been performed for experiments I and II. The authors feel that tests such as the more conservative Scheffe, Dunnett's[4] or Duncan's New Multiple Range Test are appropriate. With more replications of the experimental procedure such tests will be performed between individual pairs of groups to substantially increase the statistical validity of the results.

References

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