Urol Res (1999) 27:219–223

ORIGINAL PAPER

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Whole-body microwave exposure emitted by cellular phones and testicular function of rats

Received: 27 July 1998 / Accepted: 7 December 1998

Abstract This study investigated whether there are adverse effects due to microwave exposure emitted by cellular phones in male rats. Eighteen Wistar Albino rats were separated into three groups, a sham group and two experimental groups. The rats were confined in Plexiglas cages and cellular phones were placed 0.5 cm under the cages. In the first experimental group, cellular phones were in standby position for 2 h. In the second experimental group, phones were turned to the speech position three times each for 1 min duration over 2 h. Rats in the first and second experimental groups were exposed to microwaves emitted by phones for 2 h/day for a duration of 1 month. After the last exposure the rats were killed. Brain, eyes, ears, liver, heart, lungs, stomach, kidneys, testes, small and large intestines and skin of the rats were observed histologically. The decrease of epididymal sperm counts in the speech groups were not found to be significant ($P > 0.05$). Differences in terms of normal and abnormal sperm forms were not observed ($P > 0.05$). Histological changes were especially observed in the testes of rats of the speech groups. Seminiferous tubular diameter of rat testes in the standby and speech groups was found to be lower than the sham group ($P < 0.05$). Rectal temperatures of rats in the speech group were found to be higher than the sham and standby groups ($P < 0.05$). The rectal temperatures of rats before and after exposure were also found to be significantly higher in the speech group ($P < 0.05$). Specific absorption rate (SAR) was determined as 0.141 W/kg.

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Key words Reproductive function · Testes · Microwave · Cellular phones

Introduction

Since the earliest investigations into biological effects of electricity, time-varying fields, and radiofrequency (RF) radiation, scientists and physicians have worried about their potential hazards. Recently public concern has been focused on the potential adverse health effects of RF and microwave (MW) fields, which are used extensively in telecommunications. Special attention has focused on potential hazards of RF microwaves on cellular phone users.

Cellular phones (CPs) operate at 800–900 MHz [13]. Cellular phones may be classified as analog or digital depending on the modulation scheme employed. The analog system is called the advanced mobile phone system (AMPS). On the other hand, digital cellular phones are operated under various standards such as GSM (global system for mobile communication) and digital AMPS (DAMPS). All the systems developed for cellular phones transmit encoded, digitized information using some form of phase or frequency modulation [19].

Partial or whole-body exposure of animals to RF radiation may lead to a variety of changes in tissues. Changes in the different tissues have occurred depending on the exposure conditions, species, and histological parameters. It has been shown that prolonged, repeated exposure to electromagnetic fields (EMF) from television, microwave ovens, hand-held cellular phones, etc. without any EMF protection may produce some adverse effects in the human body [18]. Penafiel et al. [19] showed that the radiation from TDMA (time division multiple access) digital cellular phones can cause significant changes in ornithin decarboxylase activity, which is essential for cell growth and DNA synthesis. Röschke and Mann [21] did not detect any difference in the awake electroencephalograms (EEG) of subjects exposed to radiation emitted by cellular phones. Cain et al. [4] re-

ported that repeated exposures to an RF field did not influence tumor promotion in vitro [4]. Jensh [13] reported that animals exposed to 915 MHz did not exhibit any consistently significant alterations histologically. Saunders and Kowalczuk [22] reported that no difference was seen between the morphology of testes exposed to acute far field MW exposure at 2.45 GHz and sham exposure. Some authors also showed an increase in temperature and some adverse effects from 2.45 GHz MW exposure. Andrea et al. [2] did not observe significant differences between brain, heart and liver tissue of irradiated and control animals. Moreover, the adverse effects have been shown of acceptable MW radiation leakage on brain and eyes of rats [11, 12]. Seze et al. [24] showed that GSM radiocellular telephones do not disturb the secretion of anterior pituitary hormones in humans. Cleary [6] reported that RF radiation reduced the fertilizing capacity of sperm. Cleary also suggested that RF radiation and heat may have an additive or synergistic effect on reducing the fertilization capacity of mammalian spermatozoa. Moreover McRee et al. [17] found that sperm count and reproductive capacity decreased after MW exposure during embryogeny. On the other hand, effects such as so-called electrophobia have been reported [1]. The purpose of this study is to investigate the histological changes originating from MW emitted by cellular phones.

Material and methods

This study was approved by Scientific and Ethics Committee of the Medical Science Application and Research Center of Dicle University (DUSAM). Four cellular phones produced by "X" company were used in this study. The cellular phones were commercially available GSM radiotelephone handsets. The field parameters of these cellular phones are carrier frequency 890–915 MHz, 217 Hz modulation frequency, 2 W maximal peak power. Eighteen Wistar albino rats (weight 195–250 g) were caged and fed standard pellet food during the study. The rats were obtained from DUSAM. The rats were separated into three groups of 6, one control and two experimental groups [control group (sham exposed), standby group, and speech group = 6]. The rats were confined in Plexiglas cages specially designed for this study and the cellular phones were placed 0.5 cm under cages. In the first experimental group, CPs were in standby position for 2 h. In the second experimental group, phones were turned to the speech position for three min periods in 2 h. Rats in the first and second experimental groups were exposed to MW emitted by the phones for 2 h per day for a duration of 1 month. Rectal temperatures of all rats in this study were measured weekly (approximately four times during the study) by a special thermometer (Jenway 2103, UK). The rectal temperature values of each rat were calculated to find the average rectal temperature. The rectal temperature of rats in the sham exposed and experimental groups were compared statistically. The rectal temperatures before and after exposure were also measured in all groups. On the last day of the study, epididymal sperm counts were noted and evaluated morphologically. Immediately after the last exposure all the rats were killed with a lethal dose of pentobarbital intraperitoneally and their brain, eyes, ears, liver, heart, lungs, stomach, kidneys, testes, small and large intestines and skin tissues were removed for histopathological examination. The removed organs were bisected and fixed in a solution of 10% formaldehyde. The tissues were then embedded in paraffin wax, sectioned, and finally stained with hematoxylin-eosin. Histological assessments were done with a light microscope. The mean seminiferous tubular diameter was calculated for each testis. This was done by averaging the diameters of eight round seminiferous tubules randomly selected in each tissue section. Seminiferous tubular diameter of rat testes was measured and the tissues were examined histopathologically by using a testicular biopsy score count (Table 1). The average specific absorption rate was measured by using a non-interfering temperature probe technique [7]. All results in the study were analyzed by the Mann-Whitney *U*-test.

Examination of epididymal sperm counts and morphology

The left cauda epididymis of rats were homogenized with 0.5 M phosphate buffer (K_2HPO_4, KH_2PO_4, pH 7.4). The homogenate was diluted with sperm count solution. Sperm numbers per milliliter were determined by using a hemocytometer [6, 12, 16]. To determine normal and abnormal sperm forms, two slides were prepared for each rat and stained with hematoxylin-eosin. Two hundred spermatocytes per slide were scored and sperm forms were evaluated according to this score [9, 10, 15].

Results

We were studying not only the effects of MW on testes but also brain, eyes, ears, liver, heart, lungs, stomach, kidneys, small and large intestines and skin of rats. At the end of the study we observed that the testicular effect was predominant. The decrease of epididymal sperm counts in the standby group and particularly in the speech group were not found to be statistically significant relative to the sham exposed group ($P > 0.05$). The results of epididymal sperm counts in all groups are given in Table 2. No difference was observed between

Table 1 Evaluation of testicular biopsy score count by Johnson scoring [14]

Score	Description
10	Complete spermatogenesis with many spermatozoa
9	Much spermatogenesis, but germinal epithelium disorganized with marked sloughing or obliteration of lumen
8	Only a few spermatozoa present (< 5 to 10)
7	No spermatozoa but many spermatids present
6	No spermatozoa and only few spermatids present (< 5 to 10)
5	No spermatozoa, no spermatids but several and many spermatocytes present
4	Only few spermatocytes (< 5) but no spermatids or spermatozoa present
3	Spermatogonia are the only germ cells present
2	No germ cells, but Sertoli cells present
1	No cells in tubular section

Table 2 Statistical evaluation of epididymal sperm counts in control, standby and speech groups (control group = sham group)

Groups	*n*	Mean ($\times 10^6$/ml)	Median ($\times 10^6$/ml)	SD (±)	Compared groups	*P*
Control	6	236.3	232.5	35.3		
Standby	6	219.0	222.5	32.4	Control –Standby	> 0.05
Speech	6	209.8	204.0	41.7	Control –Speech	> 0.05

Table 3 Statistical evaluation of sperm morphology (normal and abnormal sperm form percentages) in the control, standby and speech groups (control group = sham group, *sp* spermatocyte)

Groups	Control		Standby		Speech	
	normal form sp (CNF)	abnormal form sp (CAF)	normal form sp (StNF)	abnormal form sp (StAF)	normal form sp (SNF)	abnormal form sp (SAF)
n	6	6	6	6	6	6
Mean	85.7	14.2	88.7	11.2	84.0	15.9
Median	85.2	14.7	89.7	10.2	83.8	16.1
SD (±)	1.9	1.9	3.7	3.7	3.7	3.7
Compared groups	CNF–StNF		$P > 0.05$	CAF–StAF		$P > 0.05$
	CNF–SNF		$P > 0.05$	CAF–SAF		$P > 0.05$
	SNF–StNF		$P > 0.05$	SAF–StAF		$P > 0.05$

Table 4 Observed seminiferous tubular diameters and their statistical results in rat testes (control group = sham group)

Groups	*n*	Mean (μm)	Median (μm)	SD (±)	Compared groups	*P*
Control	6	259.6	265.2	19.3	Control –Standby	>0.05
Standby	6	260.7	262.5	8.9	Standby –Speech	<0.05
Speech	6	202.6	205.2	8.4	Control –Speech	<0.05

Table 5 The results of Johnson testicular biopsy score count ($n = 6$). See Table 1 for classification

Score no	1	2	3	4	5	6	7	8	9	10
Control	–	–	–	–	–	–	–	–	–	6
Standby	–	–	–	–	–	–	–	1	1	4
Speech	–	–	–	–	–	–	–	2	2	2

sham and experimental groups in terms of normal and abnormal sperm form percentages ($P > 0.05$). The results of normal and abnormal epididymal sperm form percentages are given in Table 3. Measured seminiferous tubular diameter of rats in the speech group were lower than both the sham exposed and the standby groups ($P < 0.05$). The seminiferous tubular diameters of rats are given in Table 4. Histological alterations were observed in the speech group according to the testicular biopsy score count (Table 5). Rectal temperature of rats in the speech group was found to be higher than rats in sham and standby groups ($P < 0.05$). The rectal temperature after exposure was found to be higher than initial rectal temperature (before exposure) in the speech group (Table 6). The mean whole-body specific absorption rate (SAR) was determined as 0.141 W/kg. Histological alterations are shown in Figs. 1 and 2.

Discussion

The absorption of MW energy by the body results in heating. Testes, relying mainly on surface conduction for thermal control, seem likely to be an important focus of heating. In addition, during the study we observed the rats positioned prone in the cage, with the feed-point of the phone's antenna against the testes. Thus testes were relatively more exposed to MW than other organs under investigation. Sperm production in mammals is reduced by exposure to temperature a few degrees centigrade above normal body temperature, thus the testes are likely to be affected by heat generated following exposure to MW. That heat has a detrimental influence on spermatogenesis is attested to by the degeneration of germ cells in cryptorchid testes, by the temporary or permanent sterility following prolonged febrile illness and reduction of sperm counts in normal men subjected to artificial fever or occupational exposure [16].

Zarginatti and Mcleod [in 16] reported significantly lower intrascrotal temperatures (0.6 to 0.8°C) in control groups compared with patients with varicoceles. Varma and Traboulary [26] and Saunders and Kowalczuk [23] showed that 50 mW/cm^2 (1.7 GHz) MW for 30–40 min resulted in significant degeneration of seminiferous epithelium in mice. In spite of these, in Cairnie and Harding's study [5], no significant differences were noted in histo-

Table 6 The evaluation of rectal temperatures (*BE* before exposure, *AE* after exposure)

Groups	*n*	Before exposure			After exposure		
		Mean (°C)	Median (°C)	SD (±)	Mean (°C)	Median (°C)	SD (±)
Sham group (cg)	6	37.41	37.37	0.26	37.62	37.56	0.26
Standby group (stby)	6	37.74	37.39	0.33	37.81	37.86	0.53
Speech group (sp)	6	37.86	37.85	0.38	38.30	38.15	0.32
Compared groups		BE.cg–AE. cg		$P > 0.05$	AE.cg–E. stby		$P > 0.05$
		BE.standby–AE. stby		$P > 0.05$	AE.cg–AE. sp		$P < 0.05$
		BE.sp–AE. sp		$P < 0.05$	AE.sp–AE. stby		$P < 0.05$

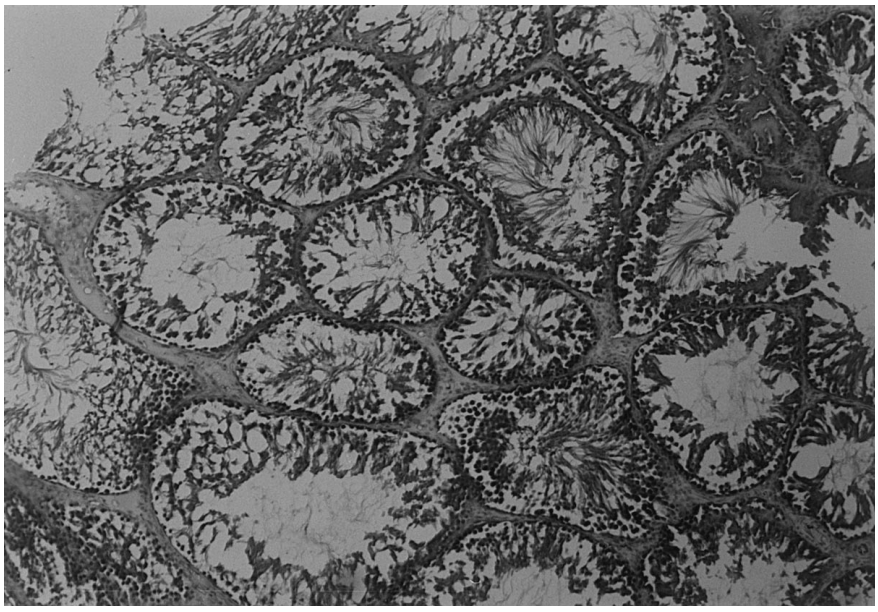

Fig. 1 Histological view of the testis of a sham group animal. Tubular lumen full of mature spermatozoa and complete spermatogenesis (original magnification ×100, H & E)

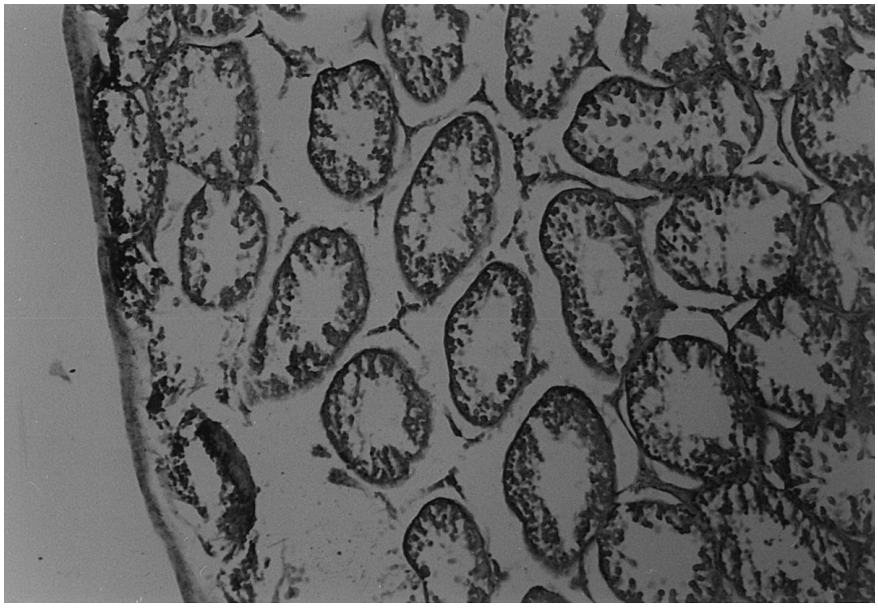

Fig. 2 Histological view of a speech group animal's testes. Note the reduction of intraluminal spermatozoa and hypospermatogenesis in 50% of seminiferous tubules (original magnification ×100, H & E)

logical evaluations of testes and sperm count or morphology between controls and animals exposed to MW for 30 days. There are few studies examining the effects of exposure to MW emitted by CP [2, 4, 13, 21, 24]. However, some of these studies focused on behavioral effects of exposure to 915 MHz MWs [2, 21].

MW can affect reproductive function by (1) a unique MW-specific non-thermal action, (2) athermal action, or (3) a combination of these mechanisms. It is commonly accepted that MW emitted by CPs are at non-thermal power density level. But there is no consensus on non-thermal exposure levels of MW in the literature [3]. In our study, chronic MW exposure of rats (SAR: 0.141 W/kg) resulted in alteration of testicular histology. The detected increased rectal temperature in the speech group exposed to the fields at an SAR of 0.141 W/kg is very surprising because it is known that irradiation with MW fields at SARs of 0.5–1.0 W/kg does not induce detectable hyperthermic changes. Therefore, it may be concluded that this is an effect of non-specific stress of non-thermal radiation emitted from CPs. Szimigielski and Gil [25] have also reported that EMFs are a non-specific biological stressor that is detected by the nervous system, and that EMFs may be a risk factor for a variety of diseases, depending on susceptibility or reactivity to stress and/or the individual genetic predisposition of the exposed host. The epididymal sperm count alteration in the speech group was not found to be significantly different to either standby or sham groups. This alteration can be attributed to the thermal action of MW because of the higher body temperature of speech group animals. Epididymal sperm count and morphological evaluation

of epididymal aspirates performed on the thirty-first day of the study revealed lower sperm counts in the speech group. The rates of morphologically abnormal spermatozoa in all groups were similar.

This application can be criticized. One could hypothesize that epididymal aspirate evaluation should be done on the sixtieth day or later from the beginning of MW exposure because it is well known that almost 60 days are required for the adult type A spermatogonia to mature into spermatozoa in rats. However, it has been shown that the early and late primary and secondary spermatocytes were the most sensitive cells to 2.45 GHz MW [23]. If this is true we would expect a decreased number of spermatozoa on the thirty-first day of the study following MW exposure because for spermatocytes to develop into spermatids takes just 14 days [16]. Another mechanism of testicular morphological alteration may be hormonal. However, Seze et al. [24] showed that GSM radiocellular telephones do not disturb the secretion of anterior pituitary hormones in humans, although the authors did not measure testosterone hormone level [24]. CPs have been used in a head-level position while in speech mode and generally hypothalamus and pituitary glands are more exposed to MW emitted by CPs. Observation of hypothalamic tissue under light microscopy revealed no differences between control and exposed animals, but under electron microscopy the presence of deep enfolding nuclei and a larger number of mitochondria in some cells were determined in Andrea and coworkers' study [2]. Our purpose in a further study will be to evaluate the effects of MW emitted by CPs on neuroendocrine and reproductive function.

The tissue effects resulting from MW exposure depend upon the power density of the source, duration of exposure, frequency of radiation, overlying materials composition of radiated tissue and its ability to dissipate heat [8]. Moreover, from many points of view the rat is an inadequate model of man in the study of non-ionizing radiation effects on the testis: its dimensions are much smaller, its scrotum is non-pendulous and its testes migrate freely through the inguinal canal between the abdomen and scrotum [5]. We used rats because of their ready availability, but difference in body size, geometry and physiological responses mean that extrapolation of these results to man is not straightforward and any such comparison should be made with great caution.

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