ORIGINAL RESEARCH

Effects of GSM-like radiofrequency on distortion product otoacoustic emissions in pregnant adult rabbits

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Abstract

Objectives: To determine the effects of 1800 MHz GSM-like Radiofrequency (RFR) on the cochlear functions of pregnant adult rabbits by Distortion Product Otoacoustic Emissions (DPOAEs).

Methods: Eighteen 13-month-old pregnant and eighteen 13-month-old non-pregnant New Zealand White rabbits were studied. They were randomly divided into four groups. Nine pregnant rabbits (Group 2) and nine non-pregnant rabbits (Group 4) were exposed to 1800 MHz GSM-like RFR 15 min daily for 7 days. Nine pregnant (Group 1) and nine non-pregnant rabbits (Group 3) were not exposed to GSM like RFR. Cochlear functions were assessed by DPOAEs at 1.0-8.0 kHz.

Results: In all pregnant groups except 2.0 kHz, DPOAE amplitudes were not different in Group 2 and Group1. In Group 4, DPOAE amplitudes at 1.0-4.0 kHz (-1.68 dB SPL at 1.0 kHz, 3.05 dB SPL at 1.5 kHz, 2.96 dB SPL at 2.0 kHz, 1.30 dB SPL at 3.0 kHz and 12.22 dB SPL at 4.0 kHz) were lower than Group 3 (8.67 dB SPL at 1.0 kHz, 17.67 dB SPL at 1.5 kHz, 26.10 dB SPL at 2.0 kHz, 18.10 dB SPL at 3.0 kHz and 35.13 dB SPL at 4.0 kHz) ($P<0.0125$). In the pregnant group, harmful effects of GSM-like RFR were less than in the non-pregnant group.

Conclusion: GSM-like RFR caused decreases in DPOAE amplitudes mainly in non-pregnant adult rabbits. Prolonged exposure may affect the DPOAE amplitude. Recommendations are given to prevent the potential hazardous effects of RF in humans.

Mobile phones have become very commonly used throughout the world.¹ The effects of nonthermal radiofrequency radiation (RFR) of the global system of mobile communication (GSM) cellular phones have been studied mostly at the molecular level in the context of cellular stress and proliferation, as well as neurotransmitter production and localization.

GSM-like RFR interferes with gene expression during early gestation.² After exposure to 900 and 1800 MHz EMFs, produced by mobile phones, no immediate after-effects on Transient Evoked Otoacoustic Emissions (TEOAEs) or measurable hearing deterioration were seen in 30 subjects.³ Similarly, when the effects of mobile phone-like 900 MHz EMFs on cochlear function in rats were investigated,

no difference in distortion product otoacoustic emissions (DPOAE) records were reported.⁴ Parazzini M, et al¹ reported that 10 min EMF exposure at the maximum power (2 W at 900 MHz or 1 W at 1800 MHz) did not induce any change in either DPOAE generation mechanism.

OAEs are by products of cochlear function and OAE characteristics over a range of stimuli provide information about cochlear status. DPOAEs are evoked upon stimulation by two frequency tones, F1 and F2, and are generated at the frequencies that combine these primary tones due to their nonlinear interaction. Although DPOAE analysis is complex and interpretation is difficult, it has been used for advanced clinical investigations on adults.⁵ DPOAEs have been used previously as indicators of cochlear status following exposure to mobile phone-like EMFs.^{1,4}

The potential hazardous effects of 1800 MHz GSM-like Radiofrequency (RFR) on cochlear function in pregnancy is an important question that needs to be answered. We could not find any previous studies on the subject. Thus, to investigate and evaluate the issue, we planned a study in rabbits. Pregnant and non-pregnant adult rabbits were exposed to 1800 MHz GSM-like RFR and cochlear function was investigated by DPOAEs comparing pregnant and nonpregnant controls.

Materials and Methods

The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All animal procedures were performed in accordance with the approved protocol and in compliance with the principles of the Declaration of Helsinki.⁶

Animal Subjects

Eighteen 13-month-old nonpregnant and eighteen 13-month-old pregnant New Zealand White rabbits were obtained from the Laboratory Animals Breeding and Experimental Researches Center of Gazi University.

They were housed under the same conditions in a temperature and humidity controlled room ($20 \pm 1^\circ\text{C}$, $50 \pm 10\%$ relative humidity) and 14-16 h light/dark cycle conditions. Except during exposure periods, tap water and standard pelletized food were provided ad libitum.

For breeding, virgin female rabbits were placed individually with male rabbits. After mating, their pregnancies were verified by abdominal palpation ten days later. Pregnant and nonpregnant rabbits were adapted to the laboratory conditions for five days before the experiment. During the five days, quality controls were conducted to verify that the rabbits were healthy.

Pregnant rabbits, 15th – 22nd day of gestation, and nonpregnant rabbits were exposed to RFR after the adaptation period. Only one animal was placed in each cage during each RF exposure period.

Experimental Design

Nonpregnant and pregnant adult New Zealand White rabbits were randomly divided into four groups:

1. Group 1 [Pregnant- Control, RF(-)]: 9 pregnant rabbits GSM-RF not applied.
2. Group 2 [Pregnant-RFR Exposed RF(+)] : 9 pregnant rabbits, GSM-RF of 1800 MHz applied for 15min/day for 7 days.
3. Group 3 [Nonpregnant- Control, RF(-)]: 9 non-pregnant rabbits GSM-RF not applied
4. Group 4 [Nonpregnant- RFR Exposed RF(+)] : 9 non-pregnant adult rabbits, GSM-RF of 1800 MHz applied for 15min/day for 7 days.

Exposure Level and Quality Control

GSM-like 1800 MHz frequency signals were formed using a signal generator (Agilent Technologies 8648C, 9 kHz- 3.2 GHz) with the integrated pulse modulation unit and horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5 - 2.8 GHz) in a shielded room. The generated power was controlled by a spectrum analyzer (Agilent Technologies

TABLE 1: DPOAE amplitudes for the frequencies 1.0-8.0 kHz of the Groups 1-4

Groups		DPOAE Results (dB)						
		1.0 kHz	1.5 kHz	2.0 kHz	3.0 kHz	4.0 kHz	6.0 kHz	8.0 kHz
Pregnant Rabbits	Group 1	5.06±	18.71±	22.80±	19.57±	32.21±	26.14±	17.78±
	Pregnant, RF (-)	7.71	13.59	21.75	22.59	23.86	24.99	25.09
	Group 2	-2.19±	5.72±	6.93±	5.96±	16.58±	14.24±	6.27±
	Pregnant, RF (+)	15.17	20.94	22.89	17.86	22.57	27.08	26.32
Non-pregnant Rabbits	Group 3	8.67±	17.67±	26.10±	18.10±	35.13±	26.07±	18.52±
	Non-pregnant, RF(-)	9.29	19.39	19.08	19.06	22.03	24.27	29.63
	Group 4	-1.68±	3.05±	2.96±	1.30±	12.22±	4.88±	-5.78±
	Non-pregnant, RF(+)	6.48	12.80	18.99	19.69	23.50	28.52	27.04
<i>P*</i>		0.008	0.025	0.002	0.028	0.006	0.030	0.016

* Kruskal Wallis Variance Analysis. Values - mean±SD

N9320A, 9 kHz - 3 GHz) integrated to the signal generator. The signal amplitude was modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 ms), corresponding to the dominant modulation component of GSM.

An RFR Generator provided 20 dB (0.1 W) power during exposure. The signal was controlled by a spectrum analyzer connected to the signal generator. A NARDA EMR 300 and type 26.1 probe were used for measurement of the output radiation. The design of the exposure system and quality controls were performed in Bioelectromagnetics Laboratory of Biophysics Discipline of Gazi University Medical School.

DPOAE Recordings

Prior to the experimental DPOAE measurements, the ears of the rabbits were examined by otoscope and animals with external auditory canal or eardrum pathology were excluded. Animals were anesthetized both during examinations and experiments with 40 mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, USA) and 5 mg/kg xylazine hydrochloride (Rompun, Bayer, Germany) *im*. Eye-blink reflexes and respiratory rhythm were followed during the experiments.

DPOAE was generated in 72 ears by ILO 288 USB II (Otodynamics Ltd Clinical OAE System, England) cochlear emission analyzer. The acoustic stimulus consisted of two simultaneous continuous pure tones at different frequencies; F1 and F2 (F2/F1:

1.22). Intensities, L1 and L2, are expressed as 80 dB sound pressure level (SPL).

Recordings were performed in an isolated quiet environment and the rabbits were sedated and motionless with regular spontaneous breathing. Plastic tubing adapters that gave the optimal fit to the external auditory canal were attached to the emission probe and a closed cavity was formed by placing the probe into the external auditory canal. DPOAE recordings and assessments were performed at the laboratories of Physiology Discipline of Gazi University Medical School.

Statistical Analysis

A statistical packet for SPSS (Version 9.0) was used for statistical evaluation. At each DPOAE frequency (1.0-8.0 kHz), the difference between DPOAE amplitudes of Groups 1-4 were analyzed by Kruskal Wallis Variance Analysis. When statistically significant results were present, a Wilcoxon Matched-Pairs Signed-Ranks Test with Bonferroni correction was used. $P < 0.05$ was considered statistically significant.

Results

In Group 1, DPOAE amplitudes were 5.06 dB SPL at 1.0 kHz, 18.71 dB SPL at 1.5 kHz, 22.80 dB SPL at 2.0 kHz, 19.57 dB SPL at 3.0 kHz, 32.21 dB SPL at 4.0 kHz, 26.14 dB SPL at 6.0 kHz and 17.18 dB SPL at 8.0 kHz. In Group 2, DPOAE amplitudes were -2.19 dB SPL at 1.0 kHz, 5.72 dB SPL at 1.5 kHz,

6.93 dB SPL at 2.0 kHz, 5.96 dB SPL at 3.0 kHz, 16.58 dB SPL at 4.0 kHz, 14.24 dB SPL at 6.0 kHz and 6.27 dB SPL at 8.0 kHz. In Group 3 DPOAE amplitudes were 8.67 dB SPL at 1.0 kHz, 17.67 dB SPL at 1.5 kHz, 26.10 dB SPL at 2.0 kHz, 18.10 dB SPL at 3.0 kHz, 35.13 dB SPL at 4.0 kHz, 26.07 dB SPL at 6.0 kHz and 18.52 dB SPL at 8.0 kHz. In Group 4, DPOAE amplitudes were -1.68 dB SPL at 1.0 kHz, 3.05 dB SPL at 1.5 kHz, 2.96 dB SPL at 2.0 kHz, 1.30 dB SPL at 3.0 kHz, 12.22 dB SPL at 4.0 kHz, 4.88 dB SPL at 6.0 kHz and -5.78 dB SPL at 8.0 kHz. Significant differences were present at all frequencies.

Discussion

In the GSM-like RFR exposed non-pregnant group DPOAE amplitudes at 1.0-4.0 kHz were lower, and at 6.0-8.0 kHz were no different from the non-GSM-like RFR exposed non-pregnant group. In all pregnant groups except 2.0 kHz, there was no difference for DPOAE amplitudes between RFR exposed and non-RFR exposed groups.

There is limited previous information on the potential adverse effects of EMFs and mobile phones on hearing¹: adverse effects are mainly result of prolonged exposure.⁷ Parazzini, et al.⁸ investigated the internal electric and magnetic field distribution of the inner hearing system exposed to 900 and 1800 MHz. They found: 1) higher internal field strength in the vestibular region than in the auditory region, especially for the inner ear closer to the external source; 2) higher internal fields strength in the basal and apical than in the middle region of the cochlea. No previous studies have explored the effects of EMF non-ionising radiations either on the DPOAE phase gradient or on the amplitude of each DPOAE emission component.¹

In the present study, decrease in the DPOAE amplitudes after exposure to GSM-like RFR in the non-pregnant group may be related to a decrease in cochlear activity and decreased activity in the outer hair cell electromotility due to prolonged exposure.⁸ In pregnancy, although increased endolymph production

due to estrogen levels and higher corticosteroid levels⁹⁻¹¹ may protect the inner ear from the hazardous effects of RFR.

We recommend the following measures to prevent the potential hazardous effects of RF in humans: 1. Avoid prolonged use of mobile phones. 2. Avoid near exposure to GSM-like RF and use ear-phones for talking with mobile phones. 3. Encourage non-continuous or temporary RF exposure.

We conclude that long-term exposure to GSM-like RFR may cause decrease of DPOAE amplitude. In pregnancy, increased inner ear volume may help to reduce the damage. This study shows only the early effects of GSM-like RFR. Future studies should investigate the late effects of GSM-like RFR.

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