Effect of exposure to radio frequency radiation emitted by cell phone on the developing dorsal root ganglion of chick embryo: a light microscopic study

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ABSTRACT

With an ever increasing number of cell phone users since late twenty first century, magnitude of the problem of exposure to radiation emitted by cell phone is self evident. Extensive research had been devoted to incriminate or absolve it as a health hazard. Radiofrequency radiation emitted by cell phone had been stated to be a potent carcinogen, cytotoxic, genotoxic, mutagenic and neurobehavioral teratogen. Its effect on the brain had been a subject of extensive research evidently due to its proximity to the user's brain. While considering the biological effects of radiofrequency radiation, its intensity, frequency and the duration of exposure are important determinants. Nevertheless the results of these different studies have not been unequivocal. Considering the contradictory reports, the present work was undertaken to study the effect of such an exposure on the developing neural tissue of chick embryo. The processes of cell division and differentiation are fundamental to the development of any living being and are a sensitive index of any insult sustained at this stage. Neurons of dorsal root ganglion were selected for the present study as these ganglia were fully differentiated as early as fourth day of embryonic life. By varying duration of exposure, the embryos were exposed to different doses of radiation, sacrificed at different periods of incubation and subjected to histological processing. On light microscopic study it was observed that developing neurons of dorsal root ganglion suffered a damage which was dose dependent and persisted in spite of giving the exposure-free period between two exposures.

Keywords: Cell phone, radiofrequency radiation, biological tissues, chick embryo, dorsal root ganglion

INTRODUCTION

Phenomenal increase in the number of cell phone users since late twenty first century has resulted in an exposure to radiofrequency radiation (RFR) emitted by cell phone becoming a matter of great concern for the population in general and the cell phone users in particular. The concern regarding its effects on the human body led to an extensive research involving epidemiological studies, in vivo studies in mammals, chicks and other lower animals, in vitro studies in cells,[1] tissues[2] and organs.[3] From the above studies some extremely hazardous effects have been highlighted. The adverse effects which had been reported vary from those at the molecular level resulting in an increase in single and double strand DNA breakages,[4] increased incidence of aneuploidy,[5] change in ornithine decarboxylase activity,[6] renal tubular damage,[7-9] increased risk of brain tumours,[10] disruption of learned behavior,[11] dysaesthesia[12] to increased chick embryo mortality.[13,14]

Conversely others have failed to detect any adverse biological effects as evident from the following reports. No change in the peripheral blood parameters was noted on exposure to RFR.[15] Its effect as an initiator or accelerator of tumours was not proved.[16] No change in the reproductive function of testes in rats was observed on exposure to RFR.[17] Cultured human lymphocytes exposed to RFR did not show any increase in the incidence of chromosomal aberrations and sister chromatid exchanges.[18] Studies were also performed to determine whether exposure to RFR could act as a neurobehavioral teratogen, the results of which had been conflicting. In some of the studies the resulting damage to the tissues were stated to be due to hyperthermia induced by the exposure to a high frequency radiation.[19] Objective of the present study is to determine the sensitivity of the embryonic nervous tissue i.e. the neurons of the developing dorsal root ganglion to radiation emitted by cell phone in chick embryo at the histological level.

MATERIALS AND METHODS

Fertile hen eggs (Gallus domesticus) were incubated in three batches. Each batch consisted of 18 eggs.

Control group: From every batch, nine eggs were incubated in a standard egg incubator at 37 ± 0· 5°C and 50-55 % humidity. They were treated as control.

Exposed group: Remaining 9 eggs were incubated under similar conditions of temperature and humidity as that of control group in a special incubator having an arrangement at its roof for mounting the cell phone. The body of incubator was made of a specific heat resistant nonmetallic material (wood) to avoid any internal reflection of radiation emitted by the cell phone. Radiation exposure duration meter was incorporated to measure the cumulative time of cell phone radiation. Meter started adding time as soon as cell phone started emitting radiation with each outgoing signal. A small fan was installed inside the incubator to keep the air circulating and prevent the formation of hot spots inside the incubator. This incubator was pre-tested for temperature control and humidity and was found to be on par with the standard egg incubator.

A standard cell phone hand set with a frequency bandwidth of 900 MHz, power of 2 Watt and Specific Absorption Rate (SAR) of 0.37 W/Kg. as standardized by the manufacturer for the said handset was used for radiating the embryos.

Exposure to radiation: Nine eggs from each batch were incubated in the specially designed incubator. Eggs were given radiation in sessions lasting half an hour each. The sessions were spaced at 12 hours interval, to give an exposure-free interval to see if the damage was reversible. The first session was at 12 hours from the starting of incubation.

For giving desired dose of exposure to radiation, the following procedure was adopted. The cell phone was placed in the incubator immediately after dialing a particular number. The cell phone started emitting signal which was recorded by radiation exposure duration meter. This was followed by a silent interval during which there was no emission of radiation. Silent interval was followed by an automatic redialing. After the redialing was complete, cell phone again started emitting signal which again activated the radiation exposure duration meter (REDM) to record the time of emission of radiation. Thus cumulative time of exposure to radiation in one session was recorded by meter (REDM). In each session at an interval of every 12 hours, the dialing procedure was repeated till the exposure for desired duration (half an hour) was accomplished.

Exposed groups of 1st, 2nd and 3rd batches received 4 hrs of exposure in 8 sessions, 5 hrs of exposure in 10 sessions and 6 hrs of exposure in 12 sessions respectively.

Both the control and the exposed groups of three batches were sacrificed at the completion of 6th, 8th and 10th day of incubation respectively.

The embryos were examined for viability and congenital anomaly if any. Only those embryos which were alive and apparently normal were taken for study. Embryos were chilled to death and then subjected to routine histological processing and paraffin embedding. Blocks were made in such a way that the whole embryo was cut in coronal sections. 5 µ thick sections were cut. Sections were stained with H&E as a routine stain, cresyl fast violet as a special stain for nervous tissue and studied under light microscope.

OBSERVATIONS

Sections of control embryos: In sections stained with Cresyl violet, dorsal root ganglia in the embryos of all the three batches were seen as distinct, well demarcated structures surrounded by a thin capsule. Dorsal nerve root was seen extending between the ganglion and the spinal cord (Fig.1). Dorsal root ganglion showed cells forming radiating cords from medial to lateral aspect. The cords of cells were separated from each other by wide spaces. Cells showed an increase in size from medial to lateral side and from deeper to peripheral part (Fig.1,2). Most of the cells in the ganglion were rounded in shape with large rounded vesicular nuclei

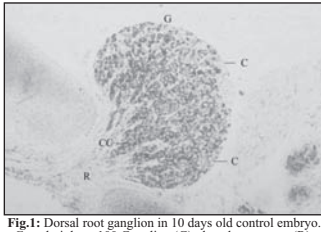

Fig.1: Dorsal root ganglion in 10 days old control embryo. Cresyl violet × 100 Ganglion (G), dorsal nerve root (R), capsule (C), cords of cells (CC).

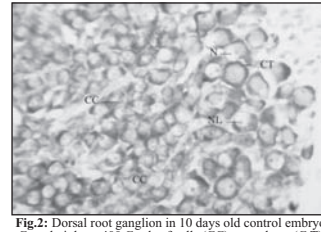

Fig.2: Dorsal root ganglion in 10 days old control embryo. Cresyl violet × 400 Cords of cells (CC), cytoplasm (C T), nucleus (N), nucleolus (NL)

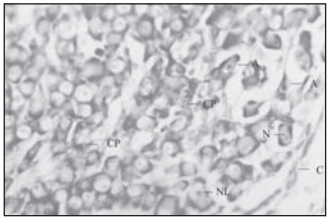

Fig.3: Dorsal root ganglion in 6 day's old exposed embryo. Cresyl violet × 400 Capsule(C), clumping of cells (CP), angulated cells (A), nucleus (N), nucleolus (NL)

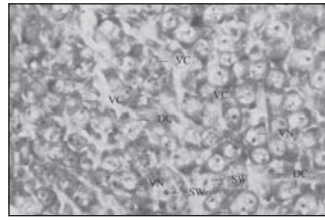

Fig.4: Dorsal root ganglion in 8 day's old exposed embryo. Cresyl violet ×400 Clumps of cells (CP), cytoplasmic vacuoles (VC), nuclear vacuoles (VN), disrupted cell membrane (DC), and swollen nucleolus (SW).

showing distinct rounded nucleoli. The peripheral cells were seen as discrete, large, rounded cells. In some of the cells, nuclei were centrally located surrounded by a peripheral ring of cytoplasm, whereas in others, nuclei were eccentric making the cytoplasm look like a crescent

Sections from exposed embryos: In the exposed embryos of all the three batches, the dorsal root ganglion was seen as a well demarcated structure surrounded by a thin capsule as in case of control embryos in 6 days old embryos (Fig.3) the arrangement of cells forming radiating cords was seen to some extent. The cells were crowded forming irregular clumps in the deeper as well as peripheral part. In the clumped areas cell boundaries were indistinguishable. In the deeper part of ganglion cells were mostly rounded in shape. In Cresyl violet stained sections, cytoplasm was stained violet and formed a peripheral ring in some and a crescent in others. The cells showed large rounded nuclei with distinct nucleoli. At the periphery of the ganglion, many cells were angulated and irregular in shape and did not show remarkable difference in size from those in the medial and central part of the ganglion. Cell margins were disrupted in some of the cells. In many cells nuclei were irregular with indistinct or disrupted nuclear margins. In some of the peripheral cells, nucleoli looked swollen and some what irregular whereas in others the nucleoli were indistinct or absent. Interstitial spaces were widened in many areas.

In 8 days old embryos (Fig. 4) the arrangement of cells did not follow any definite pattern. There was clumping of cells in many areas. The cells were seen to be studded with vacuoles which made the boundaries of many cells indistinct (Fig. 5). Some of the neurons were rounded in shape while many others showed irregular margins. Most of the cells showed vacuolated cytoplasm making it look fragmented. Vacuoles were also seen in the nuclei of most of the neurons leading to irregularity of nuclear margins. Some of the cells showed disruption of cell wall and nuclear membrane. Many cells showed nucleoli which were swollen and irregular. Some of the

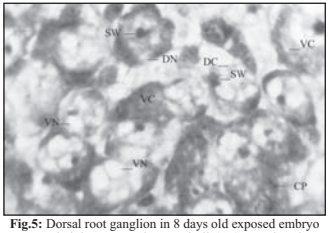

Fig.5: Dorsal root ganglion in 8 days old exposed embryo .Cresyl violet ×1000 Clumps ofcells(CP),cytoplasmic vacuoles(VC), nuclear vacuoles (VN), disrupted cell membrane(DC),disrupted nuclear membrane(DN),swollen nucleolus (SW).

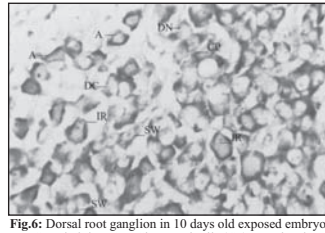

Fig.6: Dorsal root ganglion in 10 days old exposed embryo. Cresyl violet ×400 Clumps of cells (CP), angulated cells (A), irregular nuclei(IR), disrupted cell membrane (DC),disrupted nuclear membrane (DN), swollen nucleolus (SW).

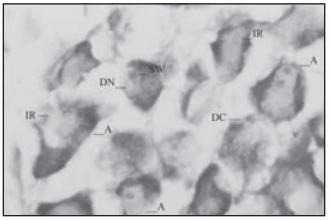

Fig.7: Dorsal root ganglion in 10 days old exposed embryo. Cresyl violet ×1000 Angulated cells (A), irregular nucleus (IR), disrupted cell membrane (DC), disrupted nuclear membrane (DN), swollen nucleolus (SW).

cells showed two nucleoli which were also irregular in shape. The changes were more extensive involving almost all the neurons.

In 10 days old exposed embryos (Fig. 6,7) the changes were extensive involving almost all the neurons. At places cells formed clumps which were more conspicuous in the deeper part of the ganglion. In some areas the cell boundaries were indistinguishable due to clumping. Most of the neurons in the peripheral part were seen to be irregular, shrunken, angulated and were apparently smaller than the deeper cells. Instead of being rounded, nuclei were irregular in shape in most of the cells. Many cells showed nuclei without nucleoli. In some cells nucleoli were swollen and irregular in shape whereas in others indistinct. Cell membrane and nuclear membrane was disrupted in many cells. Shrinkage and the angular shape of cells was the change prominently seen in most of the neurons of the ganglion. Interstitial spaces were widened adding credence to the shrinkage of cells.

DISCUSSION

In the past, many researchers have reported hazardous effects of RFR emitted by cell phone on the biological tissues.[5,7,10,11,12] On the other hand several studies have absolved it from these effects.[15,17,18] Such studies reporting deleterious effects or otherwise have used different experimental animal models like rats,[8] mice,[20] chick[21] etc. For our study chick embryo was selected as an experimental animal since the embryo was not compromised by the mother's biological system. The other points of justification for chick embryo as an experimental animal were an easy control of temperature and accurate estimation of the dose of radiation received by it.

Results of this study indicated that repeated exposures to radiation emitted by the cell phone at a frequency of 900MHz, power 2Watts, and SAR 0.37 Watts/Kg caused changes in the developing neurons of the dorsal root ganglion at the histological level. The degree of damage suffered by the neurons varied with the dose of radiation as shown by the different durations of exposure schedules. It also indicated that the degenerative changes worsened in spite of allowing an exposure-free period between the two sessions of radiation. RFR had been studied to determine whether exposure to it could cause neural teratological effects, i.e. structural damage to the nervous system of the embryo or fetus or as a behavioral teratogen. Some of the reported teratological effects in animals were stated to be due to hyperthermia induced by the exposure rather than the direct effect of radiation as many of the studies were performed at a higher range of radiofrequency radiation (2.45 GHz).[22] In the present study following factors ruled out the possibility of inducing hyperthermia of tissues.

1. Work was undertaken at a lower range of radiofrequency bandwidth (300-3000 MHz) i.e. 900 MHz, power 2 W and SAR 0.37W/Kg. which was insufficient to cause heating of tissues.
2. The temperature inside the incubator was accurately maintained at $37^0 \pm 0.5^0$ C by an electronic temperature controller.
3. Temperature inside the incubator was kept uniform by an air circulating fan installed inside. This ruled out the development of hot spots.
4. The cell phone was kept at a distance from the embryos and not touching them. The distance of the cell phone antenna (emitting maximum radiation) from the embryo (egg) ranged from 5cms (nearest) to 9.25 cms (the farthest).

The above measures ruled out the possibility of any rise in the temperature of embryos.

Not many reports were available regarding the effect of RFR on the developing neural

tissue at the histological level. Some of the studies showed that exposure to RFR had its effect on the development of the nervous system without causing heating of animals. There has been a report of the non thermal effect of exposure to Radio frequency (591MHz) radiation on the energy metabolism of rat brain cells (cerebral cortex) where the temperature was kept normal[23]. The effect was stated to be power intensity dependent, thus indicating the sensitivity of neurons to Radio Frequency Radiation. It was reported that RFR applied to the dorsal root ganglia caused a selective increase in ATF3 (activating transcription factor 3), an indicator of cellular stress, a biological effect which was unlikely to be due to thermal damage.[24] According to the above authors, the effect of RFR seemed to be selective so as to target the groups of neurons whose

axons were small in diameter like C fibers and delta nociceptive fibers.

Prenatal exposure to Radio frequency Radiation emitted by cell phone can adversely affect the development and can be a potential teratogen. The conflicting reports regarding the teratological effects of RFR could be due to different experimental animals used and the differences in frequency, power density and duration of exposure which were important determinants while considering these effects.

REFERENCES

1. Goodman R, Chizmadzhev Y, Henderson AS. Electromagnetic fields and cells. *J Cell Biochem* 1993; 51: 436-41.
2. Dovrat A, Berenson R, Bormusov E *et al*. Localized effects of microwave radiation on the intact eye lens in culture conditions. *Bioelectromagnetics* 2005; 26: 398-405.
3. Milin J, Bajic M, Brakus V. Morphodynamic reactive response of the pineal gland of rats chronically exposed to stable strong magnetic field. *Neuroscience* 1988; 26: 1083-92.
4. Lai H, Singh NP. Single and double strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int'l J Radiat Biol* 1996 b; 69: 513-21.
5. Mashevich M, Folkman D, Kesar A *et al*. Exposure of human peripheral blood lymphocytes to Electromagnetic fields associated with cellular phones leads to chromosomal instability. *Bioelectromagnetics* 2003; 24: 82-90.
6. Penafiel M, Litovitz T, Krause D, Desta A, Mullins J M. Role of modulation on the effect of microwaves on ornithine decarboxylase activity in L 929 cells. *Bioelectromagnetics* 1997; 18: 132-41.
7. Oktem F, Ozguner F, Mollaoglu H, Koyu A, Uz E. Oxidative damage in the kidney induced by 900 MHz emitted by mobile phone: Protection by Melatonin. *Arch Med Res.* 2005; 36: 350-5.
8. Pyrpasopoulou A, Kotoula V, Cheva A *et al*. Bone morphogenetic protein expression in newborn rat kidneys after prenatal exposure to radio frequency radiation. *Bioelectromagnetics* 2004; 25: 216-27.
9. Ingole IV, Ghosh SK. Cell phone Radiation and developing tissues in chick embryo-A light microscopic study of kidneys. *J Anat Soc India* 2006; 55: 19-23.
10. Hardell L, Hallquist A, Mild KH., Carlberg M, Pahlson A, Lilja A. Cellular and cordless telephones and the risk for brain tumours. *Eur J Cancer Prev* 2002a; 11: 377-86.
11. Hocking B. Preliminary report: Symptoms associated with mobile phone use. *Occup Med* 1998; 48: 357-60.
12. Hocking B, Westerman R. Neurological abnormalities associated with mobile phones. *Occup Med* 2000; 50: 366-8.
13. Grigor'IuG. Biological effects of mobile phone electromagnetic field on chick embryo (risk assessment using the mortality rate). *Radiats Biol Radioecol* 2003; 43: 541-3.
14. Ingole IV, Ghosh SK. Exposure to radio frequency radiation emitted by cell phone and mortality in chick embryos(Gallus domesticus). *Biomedical Research* 2006; 17 (3): 205-10.
15. Dasdag S, Akdag MZ, Ayyildiz O, Demirtas OC, Yayla M, Sert C. Do cellular phones alter blood parameters and birth weight of rats? *Electro Magnetobiol* 2000; 19: 107-13.
16. Santini R, Hosni M, Deschaux D, Pacheco H. B16 melanoma development in black mice exposed to low level microwave radiation. *Bioelectromagnetics* 1988; 9: 105-7.
17. Dasdag S, Akdag MZ, Aksen F *et al*. Whole body exposure of rats to microwaves emitted from a cell phone does not affect the testes. *Bioelectromagnetics* 2003; 24: 182-8.
18. Zeni O, Romano M, Perrotta A *et al*. Evaluation of genotoxic effects in human peripheral blood leucocytes following an acute in vitro exposure to 900 MHz radiofrequency fields. *Bioelectromagnetics* 2005; 26: 258-65.
19. Heynick LN, Polson P. Radiofrequency radiation and teratogenesis: A comprehensive review pertinent to Air Force operations. Armstrong Laboratory, Brooks AFB, TX, Report AL/OE-TR-1996-0036, June 1996.
20. Magras IN, Xenos TD. RF radiation-induced changes in the prenatal development of mice. *Bioelectromagnetics* 1997; 18: 455-61.
21. Espinar A, Piera V, Carmona A, Guerrero JM. Histological changes during development of cerebellum in the chick embryo exposed to a static magnetic field. *Bioelectromagnetics* 1997; 18: 36-46.
22. Cobb BL, Jauchem JR, Mason PA *et al*. Neural and behavioral teratological evaluation of rats exposed to Ultra-Wideband Electromagnetic fields. *Bioelectromagnetics* 2000; 21: 524-37.
23. Sanders AP, Joines WT, Allis JW. The differential effect of 200, 591 and 2450 MHz radiation on rat brain metabolism. *Bioelectromagnetics* 1984; 5: 419-33.
24. Hamann W, Abou-Sherif S, Thompson S, Hall S. Pulsed radiofrequency applied to dorsal root ganglia causes a selective increase in ATF 3 in small ones. *Eur J Pain* 2006; 10: 171-6.