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EFFECTS OF MICROWAVE RADIATION ON HUMAN DNA DEGRADATION

RAHI G.S.[1*], LODHI K.[2], GRIER R.[3], ADAMS J.[1] AND TORREZ A.[2]

[1]Department of Chemistry and Physics, Fayetteville State University, Fayetteville, NC 28301 USA.
[2]Department of Biological Sciences, Fayetteville State University, Fayetteville, NC 28301 USA.
[3]Atlanta Metropolitan State College, Atlanta, GA 30310 USA.
*Corresponding Author: Email- grahi@uncfsu.edu

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Abstract- Studies were conducted to evaluate effects of non-ionizing electromagnetic radiation on human DNA. In the preliminary part, trials were done to standardize the technique of irradiating DNA samples with 2.45 GHz microwaves as a function of time. Based on results of thermal patterns obtained from these trials, DNA samples obtained from blood were exposed for periods of 1, 3, 5 and 7 minutes. The changes in DNA bio-molecules were investigated by employing forensic DNA STR markers to ultimately quantify and produce a genetic profile. The experiments were replicated in triplicate. Temperature in the medium containing DNA samples reached as high as 44, 78, 96 and 97°C, respectively for 1, 3, 5 and 7-minute exposure periods. However, the samples got exposure to temperature above 90°C only after about 4 minutes. Evaluation of energy absorption resulting from microwave radiation exposure indicated that energy associated with thermal motion of molecules varied between 0.0273 and 0.0318 eV for irradiation periods of 1 to 7 minutes. The DNA samples for 5 and 7-minute exposure time periods were subjected to a net thermal power generated (about 0.1242 watt) that was more than six times of that generated in one minute exposure period (about 0.0182 watt) relative to ambient temperature. However, non-thermal and thermal energy absorption due to microwave radiation for a maximum period of 7-minute exposure did not appear to reach a threshold level to cause any permanent and systematic breakdown of DNA molecules as indicated by not any significant change in peak/height ratios in STR loci between irradiated and control samples.

Keywords- microwaves, radiation effects on DNA, STR, radiation absorption, thermal energy.

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Introduction

Effect of electromagnetic field induced by radio frequency has been attracting attention of scientists for quite some time. It is a well known fact that electromagnetic fields do have deleterious effect on living beings (including humans) if the field intensity reaches beyond a certain level. That is why safe standards of radiation exposure have been established and the electronic devices have to pass these standards. However, information reported in scientific literature on deleterious effects of radio frequency radiation has been controversial. The experimental data permeating to biological effects from this kind of radiation are fragmentary and inconsistent in many ways. Moreover, the biological significance remains ambiguous as to exactly how biological effects are resulting in adverse health impacts in people. Recent warning from World Health Organization (WHO) suggested that radiation from cell phones can cause cancer. However, WHO also assured consumers that no adverse effects had been established.

One of the ways to evaluate the effects of radiation on humans is to investigate how radiation damages human deoxyribose nucleic acid (DNA). Some studies have shown DNA damage at frequencies even lower than that used in cell phone handsets. But the others have argued that radio waves do not possess enough energy that can break the weakest chemical bonds in the genetic molecules. However, not much information is available on the cumulative effect from long and frequent use of the devices that produce electromagnetic fields comparable to those in radio frequency range.

Exposure of tissues to radio frequency radiation can cause thermal and non-thermal effects in ions, molecules, or cells. Marguet and Forterre [1] and Kuo, et al. [2] have shown that heating causes degradation of plasmid DNA. Phillips, et al. [3]; Aitkeni, et al. [4]; Lieber [5]; and Lai and Singh [6] have reported on breakdown of and damage done to DNA when exposed to low level electromagnetic fields. It has been reported that radio frequency radiation induced damage in DNA cannot be attributed to thermal effects

alone [7]. Basic premises used in designing these studies were: only the electric component of the electromagnetic waves affect the DNA molecules since they do not contain any kind of ferromagnetic material and direct exposure of DNA to radiation with relatively higher frequency (higher than that used in cellular phones) would serve as a better indicator of any damage done to the biomolecules due to non-thermal and thermal effects. Electronic devices such as cell phones work more or less like low-powered microwave ovens.

These studies were conducted to study energy relations and to evaluate effect of non-ionizing radiofrequency radiation on non-thermal and thermal breakdown of human DNA molecules in collected samples when they are exposed to microwaves for different periods of time. Results from these studies would help in understanding and evaluating any thermal or non-thermal impact (cumulative or otherwise) that the electromagnetic radiation exposure might have on human DNA caused by use of electronic devices or gadgets including microwave ovens.

Materials and Methods

In the preliminary part of these studies, trials were conducted to standardize technique to irradiate DNA samples in a conventional house-hold microwave oven using 2.45 GHz frequency. The DNA samples were put in 2.0-mL nalgene vials (Nalgene Labware) which were placed in beakers containing 500mL of water. The sample containing beaker was placed at the marked central location under the magnetron in the oven for better and uniform absorption of radiation. DNA molecules, besides getting exposure to electromagnetic field, were also subjected to elevated temperatures resulting from dielectric heating of water. This technique of holding samples in beaker with water was used for efficiency and ease to expose DNA molecules to microwave radiation and to control and maintain resulting temperature around the samples without overheating the oven.

Extraction and Quantitation

Whole blood samples were taken from 3 volunteers and mixed with heparin to prevent coagulation. The blood samples were spotted on Bloodstain cards (VWR International, LLC) were air dried for 1-2 hours at room temperature and then stored at 4°C. All Bloodstain cards were used for DNA extraction. Bloodstain cards were first pre -treated for extraction with 500 µL of digest buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS and 100 µg proteinase K) and incubated at 56°C for a minimum of three hours. Subsequently, DNA was extracted from each sample using phenol: chloroform: isoamyl alcohol (25:24:1) (17). The extracted DNA was concentrated with Ultracel-YM30 column (Millipore, Billerica, MA). These samples were then quantified with the Quantifiler® Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA) using the manufacturer's recommended protocol on the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). The DNA samples were diluted to a concentration of 1ng/µL in a total volume of 500µL TE pH 7.0 buffer in Nalgene® Cryoware cryogenic 2 mL vials and stored at 4°C. Vials made of nalgene were used as sample holders for this material appeared to be most transparent to microwaves as reported before [8]. That facilitated the sample for direct exposure to radiation with the least amount of microwave radiation absorbed by the sample holder.

Microwave Exposure

The 500µL DNA samples (2-mL Nalgene vials) were capped and inserted into sample holders floating in a Pyrex glass beaker containing 500mL of dH_2O. The beaker with the DNA samples was irradiated in a household microwave oven with a 2.45 GHz frequency with close to 1000 watt output for exposure times of one, three, five and seven minutes. Immediately after irradiation, the beaker containing the DNA sample was removed from the microwave oven. The temperature of the DNA sample was measured while in the beaker with the dH_2O and the temperature of the dH_2O was also monitored. Both the DNA sample and the dH_2O temperatures were measured using a thermocouple (thermistors). The temperature of the DNA sample was subsequently measured at 30 second intervals using the thermocouple. All DNA samples were done in triplicate. Technique for temperature measurement was standardized as conventional thermometers could not be used in the microwave oven because of interaction with radiation and the sample size. Unfortunately, fiber optic thermal sensors were not available. Temperature measurements were done by using thermocouple (thermistors) which would be placed in the sample immediately after the sample is irradiated and taken out from the oven. Because of the time lag between the radiation absorption and its conversion to long wave heat radiation and concomitant highest temperature reached in the sample, inserting thermistors in samples immediately after radiation treatment were presumed to give measurement relatively closer to the actual temperature DNA molecules were subjected to.

PCR Amplification

The Polymerase Chain Reaction (PCR) reactions were prepared according to the manufacturer's recommended protocol using the AmpFlSTR®Identifiler® Kit which detects the 15 human Short Tandem Repeats (STR) and Amelogenin makers used in forensic identification. The peak heights/ratios of the 15 STR loci and Amelogenin makers allowed for detection of possible DNA degradation. The DNA template (1ng) was removed directly from the microwave exposed tubes for PCR amplification for 1, 3, 5 and 7 minutes and control. PCR reagents were loaded into 96-Well GeneAmp® PCR System 9700 (Bio-Rad) for PCR amplification.

Detection of Alleles

Following PCR amplification, the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) was employed for electrophoretic separation of amplified products. For ABI 310 sample preparation, 24.5 µL Hi-Di™ Formamide (Applied Biosystems), 0.5 µL GeneScan™ 500 LIZ® Size Standard (Applied Biosystems) and 1 µL of PCR amplified product or AmpFlSTR® Identifiler™ Allelic Ladder (Applied Biosystems) were added to each sample. The reaction tubes were heated at 95°C for 3-min denaturation step, immediately snap-cooled on a freezer block for three minutes and then subjected to capillary electrophoresis. The samples were separated on a 47 cm x 50 µm capillary tube (Applied Biosystems).

All amplification reactions were accompanied by negative and positive controls. Amplified products were electrokinetically injected for 5 sec and fractionated on an ABI Prism® 310 Genetic Analyzer using POP4 (Applied Biosystems). Data was analyzed using a peak detection threshold of 100 relative fluorescence units (RFU)

for all dyes with GeneMapper® IDv3.2.1 (Applied Biosystems) which was used for data collection and analysis. All experiments were performed in triplicate. All STR markers are shown in [Fig-1].

Thermal energy exposure calculations were done by using absolute temperature values attained in the sample after radiation exposure and Boltzmann's constant relation [9]. Net power generated in the solution that the DNA sample was subjected to because of microwave irradiation was calculated using Stefan-Boltzmann's law [10].

The Institutional Review Board (IRB) proposal for this study was approved by the Fayetteville State University Human Rights in Research Committee (IRB # 2012-P-029).

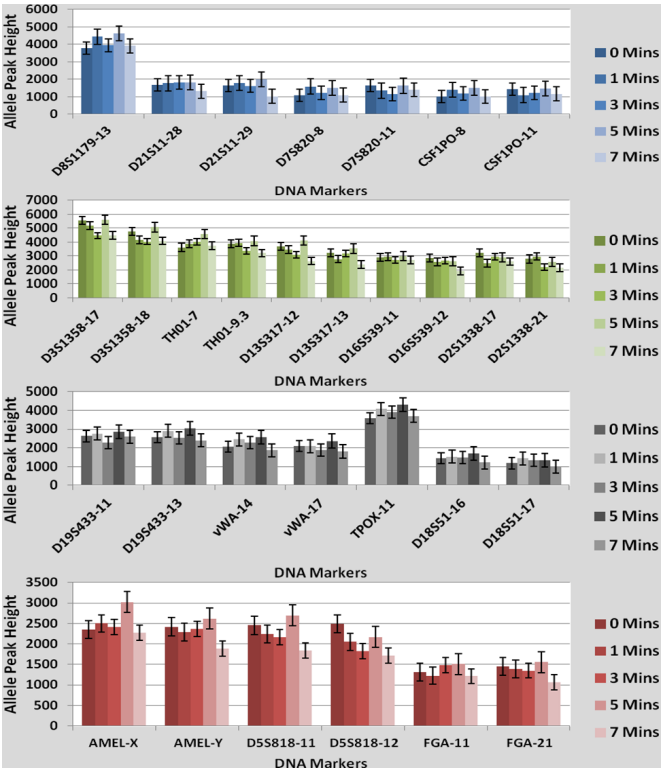

Fig. 1- Effect of Microwaves in Relation to Time

Results

The temperature of the DNA samples was measured immediately following microwave irradiation. Increased irradiation exposure (1, 3, 5 and 7 minutes) increased the temperature of the DNA sample (37.7°C, 68.7°C, 89°C and 91.3°C, respectively). The temperature of the DNA sample continued to increase after microwave irradiation indicating a time lag between exposure and energy absorption. However, the magnitude of the temperature elevation following irradiation decreased with time. These values were 17, 13, 7.8 and 6.6%, respectively for 1, 3, 5 and 7-minute exposure periods. The maximum temperature of the DNA sample occurred 60 seconds after irradiation exposure. The temperature data show that the greatest change in temperature occurred following irradiation for 1-minute period of exposure (17% temperature increase as shown in [Table-1]). Thermal energy absorption by DNA samples varied from 0.027 eV to 0.032 eV for exposure periods of 1 to 7 minutes. The 60.0-sec data in [Table-1] shows that net power generated in the vial to which DNA samples were subjected varied from 0.0183 watt to 0.1242 watt for microwave irradiation periods of 1 to 7 minutes. These values reveal that samples exposed to 5 and 7 minutes had accumulated more than 6 times the thermal energy as compared to the sample that was exposed to one minute period.

Allele frequencies of the 15 STRs and Amelogenin markers for DNA samples are shown in [Fig-1] for blood sample of one individual. Complete profiles for the 15 STR loci and Amelogenin makers were detected for all three DNA samples exposed to microwave radiation. However, two markers are detected where the individual is a heterozygous (D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, D18S51, D5S818 and FGA) and one marker where the individual is homozygous (D8S1179 and TPOX, [Fig.1]). There was no statistically significant change in peak heights/ratio in the STR loci between irradiated and control DNA samples. Similar pattern of results of showing no significant difference in peak/height ratio in the STR markers were obtained for blood samples of other two individuals (data not shown).

Discussion

The results in [Fig-1] indicate that exposure of DNA biomolecules to 2.45 GHz microwave radiation for a period of 1 to 7 minutes did not show any significant breakdown. Whatever damage to the molecules occurred was random and did not follow any specific pattern. It is even harder to explain if the random breakdown was thermal or non-thermal in nature. In the literature, most of the research reports attribute thermal energy to be the cause of microwave induced damage done to DNA by unfolding the proteins. However, in some studies, there is also a mention of an unexplained, non-thermal mechanism on DNA damage which has been referred as the "Microwave Effect". Unfortunately, no satisfactory explanation has been proposed for any direct microwave effect.

The microwave photons at a frequency of 2.45 GHz, used in present studies, possess approximately 1.0 x 10^{-5} eV energy (as per our calculations) whereas a weakest chemical bond in the DNA molecule would need at least 0.1 eV of energy to breakdown. Thus microwave photons lack sufficient energy to break the even the hydrogen bonds in DNA [11]. Microwaves are not capable of breaking the covalent bonds [12]. However, there are reports in the literature of microwave radiation with a frequency of 2.45 GHz degrading plasmid DNA [2].

The major contributing factor included in the microwave effect could be the reciprocating lorenze-force exerted on the uneven charge distribution of the DNA molecule [13]. However, according to research of Alexandrov, et al. [14] exposure with radiation of 10^{12} Hz frequency is needed to affect natural dynamics or composition of DNA. Still, effect of this force could not be completely ruled out. Non-thermal effects could also arise from motion of ions that are driven to and fro by an oscillating electric field. However, the energy associated with this motion is much smaller than that of energy associated with thermal motion of ions [15].

Energy associated with thermal motion of molecules in our studies has been calculated to be in the range of 0.027-0.032 eV for microwave exposure periods of 1 to 7 minutes [Table-1] which is also much less than the minimum needed to affect any chemical bond in DNA molecules. Thermal motion energy values for 5-minute and 7-

minute exposure times were almost identical. The energies related to another mechanism concerning attraction between cells in presence of electric field has been calculated to be appreciably less for radiofrequency fields [15].

Table 1- Thermal and Energy Relations for DNA samples Exposed to Microwaves for different periods of time

Exposure time (min)	Measurement time intervals (s)	Temperature (°C)	Energy of thermal motion generated (eV)	Net Power (watt)
	0	37.7	0.0267	0.0088
1	30	42.1	0.0271	0.0151
	60	44.2	0.0273	0.0182
	0	68.7	0.0294	0.0611
3	30	74.6	0.0299	0.0729
	60	77.7	0.0302	0.0792
	0	89	0.0311	0.1043
5	30	93.3	0.0315	0.1142
	60	95.9	0.0317	0.1207
	0	91.3	0.0313	0.11
7	30	95.1	0.0317	0.1188
	60	97.3	0.0328	0.1242

Thermal effects are caused by dielectric heating of the medium produced by the energy absorption from oscillating electric field. Microwave radiation is known to be absorbed by water molecules causing the water molecules to gain energy which can be measured as heat. However, there is a time lag between the radiation exposure and thermal energy conversion and absorption by water [16]. Thermal measurements done at 30.0 seconds indicate that the highest temperature in the sample solution containing DNA sample was reached at 60.0 second time interval [Table-1]. After 60.0 second temperature started declining. Thermal absorption pattern shows that the DNA samples were subjected to significantly higher temperature as the time of exposure increased as expected. However, this temperature increase was not much different between 5-minute and 7-minute exposure time interval. This is because water becomes a poorer radiation absorber with rising temperature according to Meissner and Wentz, [17]. The absolute temperature data used to calculate cumulative thermal energy absorption rate to which the DNA samples were exposed show that the net energy absorption rate for 5 and 7 minute intervals (about 0.1240 watt) were more than six times of that of one minute exposure (about 0.0182 watt) in 60-second interval measurements. Bohr and Bohr [18] reported that microwave radiation of 2.45 GHz frequency might cause proteins to unfold (denature). According to some research observations [19], the data pattern on microwave induced DNA covalent bond breakage greatly resemble the dynamics of a capacitor; first there is accumulation of energy and then a large release. This might show that a threshold temperature has reached or it might show a two-stage process where an accumulation of oxygen radicals generated by microwaves irradiation break the covalent bonds. The oxygen radicals can be produced by disruption of hydrogen bond which water molecules share with component atoms of the DNA. The oxygen radical would immediately bond to the nearest site, it might be an oxygen atom on the DNA causing covalent bond to break [19]. The DNA molecule might repair itself from this brief breakdown. But, simultaneous breakage of a large number of covalent bonds would cause the failure of the DNA molecule. It might also appear that DNA samples exposure to 90°C reaching after 4-minute irradiation time in our studies could cause unfolding of DNA molecules. However, in forensic analysis blood samples are subjected to temperatures close to boiling point of water without any DNA breakdown [20]. Our results also show that this gain in energy resulting from summative effect of temperature transferred to DNA molecules did not appear to cause any unfolding of the proteins or do any permanent damage to the chemical bonds. The results of these investigations, using an Identifiler® PCR Amplification Kit in conjunction with software GeneMapper ID 3.2.1 v to analyze the number of alleles in 15 different loci and respective peak heights in the human genome following treatment with microwave radiation showed no significant difference in the number of allele's peak heights of human STR loci between DNA samples exposed to microwave radiation and controls as shown in [Fig-1].

Investigating the deleterious effects, both thermal and non-thermal, of radiofrequency radiation exposure to living systems is important because of possible dangers of radiation emanating from microwave ovens, cellular phones, or other electronic devices. However, based on the results of present studies it can be concluded that exposure of DNA molecules for up to a period of 7 minutes did not appear to reach threshold level of energy absorption to cause any permanent damage to the molecules. In these studies microwaves with frequency of 2.45 GHz were used, whereas in cellular phones radiation with frequency of 1.8 GHz is used at much lower level of power output. It is planned to continue with these studies using longer periods of exposure, if possible to distinguish between non-thermal and thermal effects by direct heating of water and radiofrequency radiation with other available frequencies like 915 MHz used in industrial heating processes.

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