Non thermal effects of the electromagnetic waves on DNA: Study on E. coli

FATIMA JEBAI, MOHAMAD EZZEDINE, MANAL KHALIFE,
NISSRINE DAOU and RIAD HAMAMIEH
Biochemistry
Lebanese University
Hadath
LEBANON
mframmal@ul.edu.lb http://www.ul.edu.lb

Abstract: -E. coli strain TB1 are used to determine the effects of microwaves (MW) of frequency 900 MHz and an intensity of field exposure 6 V/m at ambient temperature. Our experiment was preformed by studying the effect of MW on DNA. We transformed unexposed bacteria with exposed pUC18 plasmid. We did not find any variation in the transformation ratio (100 transformants/μg DNA). We did not find any variation in the number of blue colonies (100% blue colonies). Analysis of exposed DNA with quantitative PCR technique was realized to determine the quantity of broken DNA strands after MW exposure. By comparison between exposed and control DNA no difference was observed. Electrophoresis and spectroscopic analysis of exposed DNA did not reveal any hyperchrome effect. In order to confirm our results we sequenced exposed pUC18 plasmid but again no alteration of the DNA on the molecular level was observed.

Key-Words: - Microwaves, Elecromagnetic, Mobile, DNA, Mutation, HSP

1 Introduction

During the last decade, the use of the mobile telephone showed a considerable growth in the whole world. These telephones emit electromagnetic waves, which have effects on the biological systems.These effects cause modifications that can be biochemical or physiological nature.
Since the beginning of the century, it was known that electromagnetic waves are absorbed by biological systems and at sufficiently high power, they can induce an increase in temperature[1][2]. Studies of this heating effect lead to the establishment of safety requirements and exposure limits (ANSI/IEEE C95.1/1991).
However, during the last fifteen years, there has been a more precise knowledge of the interaction between biological system and radiation; called non-thermal effect due to a lower power. The existence of these effects has caused a great controversy; many studies concerning the non-thermal effect of electromagnetic waves[3][4] were carried out as studies of the bactericidal effect; the effect on the cellular proliferation[5][6][7][8][9], genotoxic effects (DNA damage or mutations)[10][11][12]. These studies often gave contradictory results.

2 Problem Formulation

The subject of this paper was to study non-thermal effect due to long-time and low intensity field exposure. The condition of radio-exposure environment to field intensity polarization was performed to be as near as possible to the real case on the proximity of a base station emitter (BTS) in GSM system.

2.1 Material and methods

2.1.1 Bacterial strain, culture medium and condition of culture were as follows

The bacterial strain that was studied is E. coli TB1(ara,D(lac-proAB),rpsl(f80lacZDM15) hsdR. The culture media used were liquid and solid LB medium. The strain grown in Erlenmeyer (250 ml) containing 50 ml of LB then incubated at 37°C under agitation (250 rpm). The duration of

incubation depends on the phase of growth in which we want to study the bacterial strain (6 hours for a suspension in exponential phase and a night for a suspension in stationary phase).
The aim of this preclude is to take both experimental and control samples to study the various parameters.

2.1.2 MW used and protocol of exposure

The exposure has been realized on anechoic-chamber that is an isolated local from electromagnetic field. The following condition has been respected for all exposure time:

- field intensity 6 V/m
- uniform amplitude distribution
- vertical polarization of field

The protocol of exposure consists of a phase of exposure to the MW during 10 days. For each exposed bacterial suspension (experimental) another unexposed suspension (control) was observed in the same condition but without the influence of MW.

2.1.3 Bacterial transformation by exposed pUC18:

In order to study the effect of the electromagnetic waves on DNA, bacteria were transformed with exposed pUC18 plasmid using the $CaCl_2$ method. Bacterial cultures were diluted then spread over Petri plates containing ampicillin, IPTG and X-gal. After 24 hours incubation at 37°C, counting of the blue and white was carried out.

2.1.4 Spectrophotometric analysis of DNA

Extracted DNA from bacteria was diluted then the absorption spectrum (between 200 and 900 nm) was plotted. This DNA was then exposed for 10 days then the absorption spectrum was redone. Maximum absorption of DNA occurred between 260 and 280 nm.

2.1.5 Electrophoresis DNA analysis:

DNA samples where extracted from bacteria previously exposed to MW for 10 days. Those DNA along with control ones where digested then migrated on agarose gel.

2.1.6 Quantitative PCR (polymerase chain reaction)

Quantitative PCR reaction was done on pUC18 plasmid from exposed bacteria using the following primers: oligo 3': 5'TGGGGTGCCTAATGAGTGAG3' and oligo 5': 5' TGTCCTCGCTCTGCTAATCC3'. 20 PCR cycles where performed and the product was analyzed on agarose gel. The resulting DNA fragment is 701 base pairs (bp) and extends between the base number 547 and 1248 from pUC18.

2.1.7 Sequencing

Quantitative PCR product was exposed for 10 days. The amplified DNA fragment was then sequenced by an automatic fluorescent technique at USJ University in Beirut.

2.2 Results

After bacterial transformation by exposed pUC18, we have obtained 100% blue colonies.
Better understanding of the mechanism of MW can be achieved by studying its effects on DNA. To check for any conformational change in the DNA, samples were exposed to MW for 10 days. Agarose gel electrophoresis showed no difference in the migration patterns between none exposed (NE1) and exposed (E1) non digested pUC18 plasmid. No changes in the amount of supercoiled and relaxed DNA were observed. Restriction enzymes digested 100% of pUC18 in both exposed (E2) and non exposed (NE2) forms. (Fig.1). No changes in the amount of supercoiled and relaxed forms were noticed before (Fig.2) and after (Fig.3) exposition.. Restriction enzymes digested completely the plasmid. Absorption curves indicate the absence of hyperchrome effect.
These results demonstrate that DNA concentration and conformation did not change. Quantitative PCR did not show any significant difference between the quantities of polymerized DNA before and after the exposure. After sequencing of the exposed 702 bp PCR fragment we compared it with the pUC18 control sequence and found no change in the DNA sequence (Fig.4).

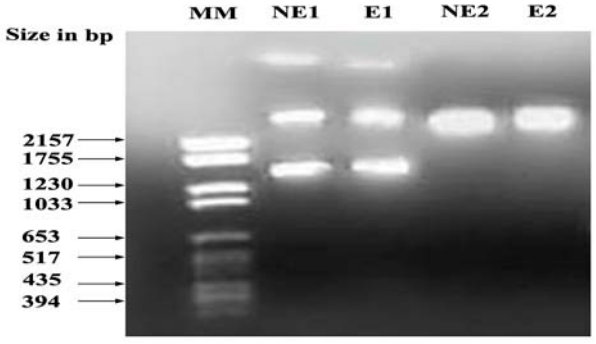

Fig.1

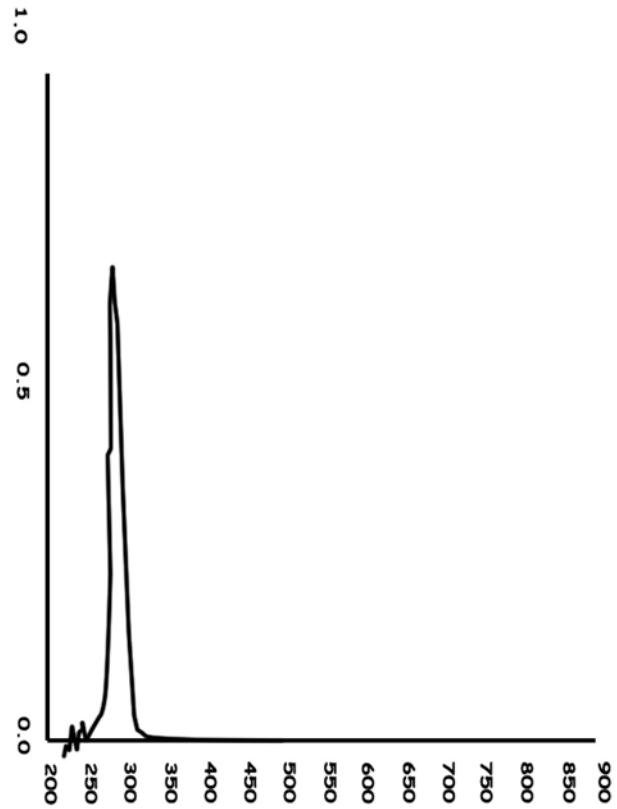

Fig.2

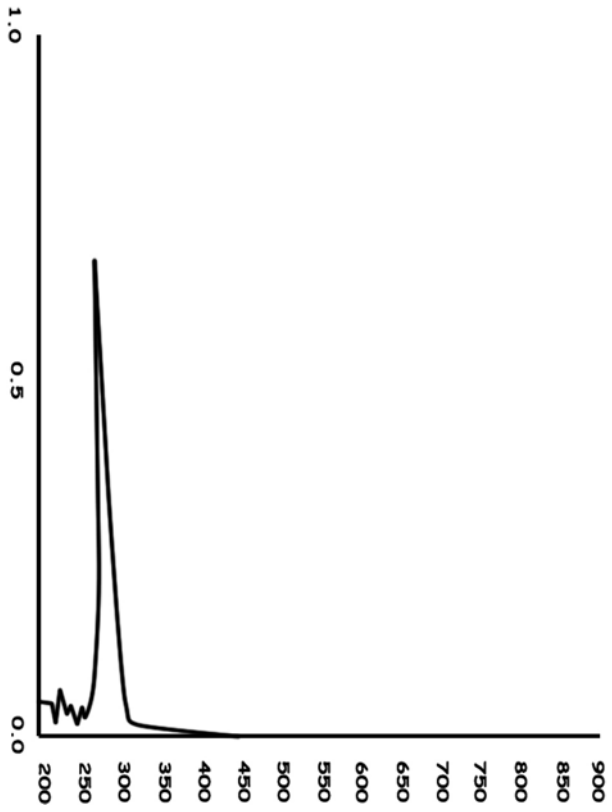

Fig.3

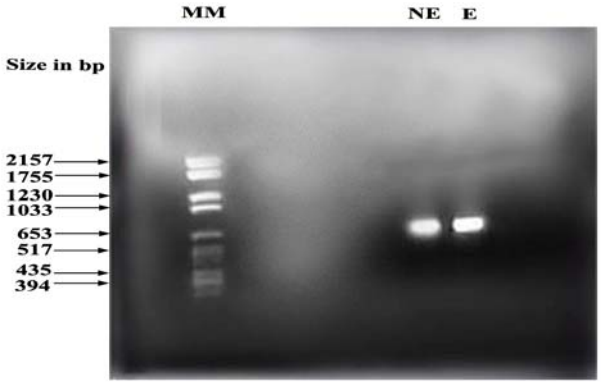

Fig.4

3 Discussion

After the series of experiments carried out in vitro (in order to clarify the effect of the MW of a base station mobile) the results obtained were more or less in agreement with the literature. These effects were applied continuously since humans are exposed daily to MW from base station mobile.

While other studies did not observe any activation of HSP[13][14] and we think that they play a role in DNA stability.
Some studies showed that there's an induction of heat shock proteins (HSP) during microwaves exposure of bacterial cells[15][16][17]. HSP are chaperon proteins which intervene during protein folding.
An increase in HSP will translate into more efficient protein folding and thus more active proteins[18][19][20][21] which lead to higher resistant bacteria.

HSP plays a role in the reparation of muted DNA. This might explain why no mutations where observed in our DNA sequence. We note that other researchers did obtain DNA mutation by using mutagenic factors[4] or oxidants like H2O2[22] during MW exposure.

Other studies showed that during MW exposure, transposition elements are activated[23].Others found that reparation proteins are also activated. This can explain why we observed no DNA mutations on the molecular level.

The study on the DNA alone shows the absence of effect on the conformation of the nucleic acid. Moreover, the absence of an effect on gene expression was shown. 100% of the pUC18 containing bacteria showed β-galactosidase activity

(blue colonies were obtained in the presence of X-gal and IPTG; no perturbation was observed at least in this gene. These results contradict King-Chuen Chow's results[24], which used the same technique used here. They found out that 2% of E. coli containing the pUC18 plasmid did not show β-galactosidase activity.

In addition, like some previous studies[25][26], no effect of the MW on DNA conformation was shown. If the DNA conformation has changed, different migration patterns on agarose gel and variations in the absorption curves would have been noted.

Other studies by Lai H. and Singh proved the existence of DNA fragmentation[27][28].
To further back up obtained results, two more experiments were performed. First, to see if exposing a pUC18 plasmid would affect the bacterial transformation, the plasmids were exposed to MW for 10 days and then used to transform E. coli. No change in the transformation percentage was detected and we obtained blue colonies in the same ratio as with control pUC18. Moreover, a DNA sequencing of an exposed pUC18 fragment was performed to determine if there is any mutation on the DNA molecular level. Comparison between pUC18 sequence and the one obtained did not show any type of mutations in the DNA sequence like in previous works[29].

4 Conclusion

By studying the effect of MW on DNA we found that DNA wasn't effected directly by the MW. We suspected that the effect was indirect and mediated by proteins. Further study of the activation of proteins by MW will give better understanding on the direct and indirect effects of MW on DNA.

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