ORIGINAL ARTICLE

Effect of long-term exposure of mice to 900 MHz GSM radiation on experimental cutaneous candidiasis

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Abstract Mobile phones communicate with base stations using 900 MHz microwaves. The current study was aimed to survey the effects of long-term 900 MHz microwave exposure of mice on experimentally induced cutaneous candidiasis. Forty inbred, male, BALB/c mice were randomly divided into four groups. Cutaneous lesions with Candida albicans were experimentally induced on the lateral-back skin of the 20 mice. One group of the diseased mice were exposed (6 h per day and 7 d per week) to 900 MHz microwave radiation, while the other groups were not exposed. Two unexposed control groups were also included. The skin lesions were regularly monitored and the live candida cell density was enumerated using the colony-forming unit (CFU) assay. The process was repeated after a one week resting interval. One week later, all mice were challenged through intra tail veins using LD90 dose of C. albicans. Mortality of the mice was recorded and the candida load of the kidney homogenates from died animals was counted. 900 MHz microwave exposed mice had 1.5 day and 3.7 day delays on wound healing in stages two. Live Candida inoculated Wave exposed (LCW) mice also showed higher yeast loads in skin lesions at days 5, 7 and 9 post inoculation. Survival analysis of live candida challenged mice showed the radiation exposed group is prone to death induced by systemic infection and candida enumeration from the kidney homogenates showed radiation exposed animals had significantly higher yeast load in the tissue. In conclusion, long-term 900 MHz radiation exposure of mice led to longevity of skin wounds and...
sustainability of the animals to systemic challenge and higher incidences of microorganisms in internal tissues.

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1. Introduction

Nowadays, electromagnetic radiations from man-made instruments, such as radio-television broadcasting and wireless telecommunications, e.g. cellphones, are the major physical contaminants in the environment. Results of numerous studies on biological impacts of the waves have already been published. During the past decades, various effects of the electromagnetic waves (EMWs) on wound healing have been studied, however, literature reports on the field are often associated with high discrepancy (Henry et al., 2008). It seems that a satisfactory explanation for the discordance relies on two main influencing factors: the vastness of the radiation parameters and the diversity of the biological systems. Radiation parameters include, but not limited to, the wave frequency, the wave intensity, continuous or discontinuous nature of the radiation and duration of the impact. The frequency of the EMWs is varied from one Hz to $10^{14}$ Hz or above. Low frequency waves carry lower energy and do not have ionizing potential, while higher frequency waves carry enough energy to ionize atoms upon impact. The intensity of any radiation is directly proportional to the intensity of radiated source and is inversely proportional to the square of the distance from the source. In addition, duration of the radiation is also an important factor (Johansson, 2009). The diversity of biological systems is the second factor influencing the outcomes of the radiation exposure. The biological systems usually are composed of eukaryotic or prokaryotic cells. The cell is comprised of diverse compartments, macro and micromolecules, membranes, as well as specialized components like signaling pathways that are means of trans ferring environmental variation signals into the cells, often into the nucleus, which plays a pivotal role in the management of different cell processes including responding to different stressors. EMWs may affect all components of the cell and the final outcome depends on the nature of both radiation and target molecules. Mobile phone handsets and the base stations, both are operating with microwaves ranging from 900 to 1800 MHz (Iskra et al., 2007). The waves are modulated by voice signals to carry the information and do not possess any direct ionizing effects on solutions or biological systems, but instead, microwaves do possess thermal and non-thermal effects that, per se, are very important for human health. Regulatory bodies in different countries have set minimum acceptable levels of radiations from cell phones or from the base stations according to the intensity of thermal effects, but exploring for non-thermal effects of the radiation is continued by many researchers to discover any possible hazardous effects of the waves on human health (Rubin et al., 2006; Belyaev and Grigoriev, 2007). Microwave exposure of the whole body leads to notable rise in the tissue temperature (Masuda et al., 1985), changes the tissue electric potential (Moulin et al., 2012; Huttenlocher and Horwitz, 2007), disturbs signaling pathways (Maskey et al., 2010), denatures critical macromolecules (Fattahi-Asl et al., 2012), vibrates and ruptures different cell membranes, and in collection, microwave exposure acts as a cell stressor that in low intensity and shorter duration may be beneficial, since it triggers cell and tissue repairing systems, but in high intensity and longer durations is inevitably hazardous for cell survival and tissue hemostasis. Microwave effects on skin wound healing have been investigated by many researchers, but usually not with wounds of infectious origin. The current study was designed to evaluate the 900 MHz microwave radiation, exactly the same as radiations from the cell phones, on curing experimentally induced cutaneous candidiasis. Different strategies have been applied to induction of cutaneous candidiasis. Ray and Sohnle (1976) studied the occlusive dressing method for induction of candida-specific infectious skin lesions. This strategy is applicable to little mice, because newborn mice have bare and well-exposed skin. Very small skin rashes are produced only on the surface layer of the skin and the inflammation is not associated with notable numbers of immune cell activation. Therefore, memory immune cells do not develop through the occlusive dressing process (Sohnle et al., 1976; Ray and Wuepper, 1976). The second way was introduced by Giger et al. (1978), which is about intradermal injection of Candida albicans used for induction of intracutaneous nodules. The advantages are possibility of temporal measurement of nodule dimensions and preparing tissue biopsies to tracking immune or migrating cells. To the best of our knowledge, the best method is scratch-induced candidiasis applied by Wu et al. (2003), who experimentally induced keratomycosis in a mouse model. In the process, an optimized suspension of live C. albicans was repeatedly applied to the bare and previously scarified skin of the mice. Surface scratching of sterilized skin with a scalpel destroys the major barrier of the skin and the yeast has the opportunity to penetrate into the skin layers. In this way, immune cells are usually stimulated against the yeast and the memory of infection is developed by the immune system (Domer and Moser, 1978; MacCallum, 2012; Sohnle et al., 1976; Wilson and Sohnle, 1986). The aim of the present study was to evaluate the effects of long-term microwave exposure of mice on the curing rate of experimentally induced cutaneous candidiasis.

2. Materials and methods

2.1. Materials

C. albicans (ATCC: 10231) was used through all stages of the study. Male inbred BALB/c mice, at 6–8 weeks of age, were purchased from Pasteur Institute of Iran and kept in a standard animal housing facility with adequate pellet and water for animal consumption. The animal study was approved by the Institutional Ethics Committee. Dexamethasone (Sigma: D1756) was used for immunosuppression. Chlorpromazine was used as a sedating agent for better restraining of the animals during manipulations. Sabouraud Dextrose (SD) (Merck:
105438), SD agar or SD broth mediums were used as culture media with chloramphenicol (Merck: 220551) as an antibiotic to prevent from undesired microbial contamination of the cultures. The radiation generator was a standard jammer with 900 MW output and 3 pieces omnidirectional antennas. All culture and isolation media and related reagents were from the university resources.

2.2. Culture conditions

*C. albicans* was subcultured (from a stock medium that had been stored at 4 °C) onto the SDA plate and incubated at 25 °C. For skin surface inoculation, *C. albicans* was further subcultured at 37 °C at least for 24 h. For preparation of the killed Candida, the yeast suspension (suspended in saline solution) was placed for 30 min in a 60 °C water bath. The samples from the preparation were inoculated on SDA plates and incubated to confirm the absence of any live candida in the solution.

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2.3. Study design and animal grouping

All stages of the study, the treatments and the measurements have been presented briefly in Table 1. According to the study proposal, 40 male BALB/c mice were randomly divided into four groups of ten animals. For experimentally inducing cutaneous candidiasis, all mice were immunosuppressed using oral administration of dexamethasone (1 mg/l in drinking water). The hairs of the flanking region of the animals were shaved and the bare skin was scratched using an upstanding bistoury. The scratched skin of the mice in group 2 was inoculated using killed *C. albicans* suspension. The same location in the groups of three and four was inoculated using live *C. albicans* suspension that had previously been incubated for 24 h at 37 °C. Only group 4 mice were exposed to 900 MHz radiation. One week after complete healing of the first stage of lesions, other lesions were induced in the opposite side of the back. The measurements for both, the first and the second, stages were the same.

### Table 1 Animal grouping and the measures (in temporal order).

| Group number | 1: N | 2: KC | 3: LC | 4: LCW |
|--------------|------|-------|-------|--------|
| Dexta-Tetra  | ✓    | ✓     | ✓     | ✓      |
| Hair cut     | ✓    | ✓     | ✓     | ✓      |
| Skin scratch | ✓    | ✓     | ✓     | ✓      |
| Killed Candida| ✓  | ✓ | ✓ | ✓ |
| Live Candida | ✓ | ✓ | ✓ | ✓ |
| Exposure to wave (900 MHz) | ✓ | ✓ | ✓ | ✓ |
| CFU determination | ✓ | ✓ | ✓ | ✓ |
| Live candida challenge* | ✓ | ✓ | ✓ | ✓ |
| CFU from kidney** | ✓ | ✓ | ✓ | ✓ |

KC, killed candida; LC, live candida; LCW, live candida inoculated; 900 MHz radiation exposed mice.

* Done after complete healing of second stage skin wounds.

** Done after survival test results recorded.

2.4. 900 MHz radiation exposure

Only group 4 mice were always kept near the radiation generator, until the end of the study (6 h per day, 7 d per week). The device was turned on to radiate 900 MHz waves with a total duration of 6 h per day.

2.5. Measurements

For cutaneous wounds, full healing of the lesions and complete removal of the yeast from lesions were evaluated using the CFU assay. Swab samples from the lesions were prepared on days of one, three, five, seven and nine and yeast loads were quantified. The wounding day was set as zero.

2.6. Microbiology (CFU assay)

2.6.1. *C. albicans* in skin lesions

For temporal monitoring and quantifying *C. albicans* in skin lesions colony forming units, the CFU assay was used. The swab samples from skin wounds were prepared on 1, 3, 5, 7 and 9 days after inoculation. The swabs were soaked for 1 h in a tube containing 1 ml of SD broth. Then, the swab was discarded and the resulting suspension was further serially diluted in four other tubes (1:10 to 1:10,000) using SD broth. The surface plate count method was used for counting grown colonies. Briefly, 50 μl from each dilution was aseptically transferred onto SD agar plate and distributed using an L-shaped glass. The inoculated plates were incubated for 24 h at 30 °C and the grown colonies were counted and recorded. The plates were further incubated up to 72 h if any colonies were not detected.

2.6.2. *C. albicans* in kidney homogenate

According to the literature, during systemic challenge *C. albicans* had a strong tendency to infect kidneys. For detection and quantification of *C. albicans* in kidney samples collected from animals died of the live candida challenge, the kidneys were aseptically excised and ground. 200 μl from each homogenized kidney sample was suspended in 800 μl of SD broth and further dilutions were prepared using the same medium. Plating and colony counting methods were the same as previously mentioned for processing the swab samples.

2.7. Live *C. albicans* challenge of mice

We assumed that our interventions including surface inoculation of *C. albicans* or long-term exposure of mice to 900 MHz radiation, potentially could enhance or weaken mice immunity against lethal dose challenge of mice with live candida. For evaluation of long-term radiation exposed mice resistance against a lethal dose challenge with live *C. albicans*, 100 μl suspension containing 2 × 10^5 cells was injected through tail vein of each mouse (totally 40 mice). The mortality of the mice was considered as a wanted event and was recorded in daily manner up to 10 days. The remaining surviving mice were also euthanized at the end of the study and the kidneys were excised, and the number of *C. albicans* in the tissue homogenates was quantified using the CFU assay.
2.8. Statistical Analysis

The data were analyzed by SPSS version 16.0 IBM statistical software. According to the nature of data, two independent samples, *T*-test or paired samples were used. Moreover, *T*-test was used for statistical comparisons. The differences among the mean values were found to be significant at \( P \leq 0.05 \).

3. Results

3.1. Cutaneous candidiasis

**Visual observations**: Results of two stages wounding have been presented and compared below. Development of skin lesions were only limited to the scratched regions of the skin, which makes further emphasis on barrier properties of the rigid dry skin against candida infections.

Daily visual observations were done at time points of 1–13 days (Fig. 1: horizontal axis) after wounding (inoculation day was considered as zero). Accumulated frequency of completely cured wounds was recorded for each group (Fig. 1: vertical axis). A wound was considered as completely cured when the deeper layers of the skin were repaired and the dead crust layer was repelled. Accumulated frequencies of the cured wounds over two stages of the study have been given in Fig. 1.

**Other visual findings**: the median cure days (defined as: the most frequent numbers of cured wounds per a day) for 4 groups of N, KC, LC and LCW in the first stage were days of 3 (with 4 cures), 3 (5 cures), 5 (8 cures) and 6 (6 cures), respectively. The results for the second stage were 3 (4 cures), 3 (5 cures), 5 (4 cures) and 8 (4 cures), respectively. That means, in the radiation exposed group (LCW) the highest numbers of cured wounds have occurred at day 6 and 8 in the first and second stages, respectively (with 2 days delay in the second stage).

Mean cure time for each group: for further elucidation of between groups differences mean cure day of wound for each group was calculated and compared. The results have been shown in Fig. 2.

3.2. Microbiology

The swab samples from cutaneous lesions were prepared on time points of 1, 3, 5, 7 and 9 days after inoculations – during the two stages of the study. Yeast load of swabs were assayed using the CFU method. The log10 number of *C. albicans* (per swab) segregated by the day of the sampling and type of treatments has been presented in Fig. 3.

3.3. Live candida challenge of all mice

With live candida challenge, we wanted to evaluate the impact of long-term radiation exposure on resistance of the animals to the lethal challenge of the yeast. For the estimation, an optimized LD90 dose of live *C. albicans* was injected through intra...
tail vein of mice in all 4 groups and any mortality was recorded
during the next 10 days. The resulting survival diagram has
been presented in Fig. 4.

3.4. Estimating C. albicans in kidney samples

Colony counting results from kidney homogenates of died ani-
mals have been given in Table 2.

4. Discussion

The impacts of electromagnetic radiation on cells and tissues
have extensively been studied before, but the studies on the
radiation effects on skin wounds with infectious origin are
rare. The current study was designed to survey 900 MHz
microwave radiation effects on healing of experimentally
induced cutaneous candidiasis in BALB/c mice model. In
order to attain tangible results: (a) wounding process was
repeated (with 1 week interval), (b) radiation exposure of mice
was set to 6 h per day, from the beginning to the end of the
study, and (c) systemic challenge of mice was carried out using
LD90 doses of live C. albicans.

Our findings showed that 900 MHz radiation exposure of
mice in the LCW group led to 1.5 days (in stage 1) and 3.7 days
(in stage 2) retardations in mean wound healing time com-
pared to unexposed mice (LC group) (Fig. 2). Also, colony
counts of C. albicans from wounds at 5, 7 and 9 days post in-
oculation revealed removal of the yeast from wounds was ineffec-
tive in the radiation exposed group (LCW) compared to in
unexposed mice (LC). Survival analysis of live Candida cha-
lenged mice (Fig. 4) showed the radiation exposed group is
prone to death induced by systemic infection and candida emu-
eration from kidney homogenates showed radiation exposed
animals have had a significantly higher yeast load in the tissue
(Table 2). In collection, long-term 900 MHz radiation expo-
sure of mice led to longevity of skin wounds and susceptibility
of the animal to systemic challenge and higher incidences of
microorganisms in internal tissues.

The aim of the current study was innovative because micro-
wave effects on duration of infection and survival of animals
have not been studied yet. Therefore, we review here other
literatures that have been published about microwave effects on wound healing and immune cells. Wound healing is a complex but highly regulated process, divided into three phases: inflammation, proliferation and maturation (Moulin et al., 2012). Through the inflammatory stage phagocytic cells, including neutrophils and macrophages, infiltrate into the injured site for mounting a host defense response and quenching inflammation through removing infective agents, necrotic and sloughy tissue. Proliferation stage is associated with rebuilding of extracellular matrix and developing new vessels that provide enough oxygen and nutrients to support fibroblast growth and granulation tissue formation. Bone marrow-derived stem cells also migrate to the site and develop into specialized cells to rebuild tissue structure. Finally, epithelial cell growth occurs to surround wound surface, named epithelialization. During maturation phase, collagen type III is replaced by collagen type I, cellular activity is reduced and the number of blood vessels decline to normal state.

An skin wound usually in literature refers to a full-thickness cutting of skin tissue that deeply destroys the skin, but our intervention through the study involved mild scratching of the surface dead layer of the skin and inoculation of *C. albicans* in order to induction of an infectious skin lesion with enough duration of wound to implement the study plane. Therefore, we supposed that the healing process in the current study mainly composed a long inflammatory stage followed by rapid proliferation and maturation stages. A thick viscous crust was usually formed on the surface of the lesion that makes the swab sampling difficult and complete healing of lesions was associated with shedding of the crust. Through inflammatory phase, *C. albicans*, as an invader, infiltrates into the skin and triggers an immune response with recalling of phagocytic cells, neutrophils and macrophages (Hahn and Sohle, 1988).

In addition to phagocytes, many different cells and many soluble and insoluble factors contribute to wound healing and microwaves may influence any of these components. Nikolaev et al. (1984) studied the stimulatory effect of 300 Hz sinusoidal current on wound healing in rat model of linear and large incised wounds. Improvement in microcirculation, reduction in inflammation, increase in proliferation and differentiation of fibroblast and epithelium and acceleration of healing process was reported. Apparently, the effects are limited to the provided conditions, because any increase or reduction in optimum frequency (300 Hz) and exposure time led to notable decrease in the beneficial effect. Natural epithelial-derived sodium currents and their role as normal controlling factors in wound healing have been discussed (Lee et al., 1993). A previous study by Stankiewicz et al. (2006) studied the effect of 900 MHz GSM microwave on human blood cells. Microwave-exposed cells demonstrated higher response to mitogens and higher immunogenic activity. Also, another conducted survey by Athanasiou et al. (2007) studied the pulsed electromagnetic fields effect on skin wounds healing. 20 min daily exposure of rats through the first 9 days led to acceleration of wound healing confirmed by histological

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**Table 2** Colony counts from kidney homogenates.

| Groups | N    | KC   | LC*  | LCW** |
|--------|------|------|------|-------|
| Mean ± SE | 6.3 ± 0.37 | 5.1 ± 0.21 | 3.8 ± 0.31 | 7.7 ± 1.1 |

Mean and standard deviation of colony counts as log_{10} CFU value per gram of kidney samples in 4 groups of mice.

* Significant differences of LC with 3 other groups, N, KC, LCW.
** Significant differences of LCW with 2 other groups KC, LC.
exposed to 900 MHz microwaves, 6 h per day over a time period of at least 2 months and then challenged with LD_{90} dose of live _C. albicans_ had a mortality rate twice higher than that of the unexposed group. We found at least one possible explanation for this tragic outcome. Enumeration of yeast load on kidney homogenates of died animals showed that the radiation exposed animals had a significantly higher candida load in the tissues compared to the unexposed mice.

Immune cells are key regulators of immunity against invading microorganisms and spontaneously produced mutated cells of the self-origin. The other study by Lyle et al. (1983) reported transitional inhibitory effect of 450 MHz radiation on cytotoxic T lymphocytes. Fesenko et al. (1999a,b) described whole body microwave irradiation effect on TNF production by peritoneal phagocytes. Microwave exposure of mice, 5 h per day more than 7 days, led to reduced TNF production that the effect persisted for 3 days post-exposure (Fesenko et al., 1999a). Fesenko in 1999 in another study demonstrated that if exposure time of mice was set to below 5 h, the radiation could not influence natural killer cell density in spleen (Fesenko et al., 1999b). The previous study reported that 900 MHz GSM waves in vitro effect on human lymphocytes. 1 h per day for 3 days exposure to 900 MHz radiation led to lower sensitivity of the lymphocytes for mitogen and the cells showed increased exposure of phosphatidyserine, a marker of cell apoptosis, in the membranes (Capri et al., 2004). In general, long-term exposure of whole body or isolated immune cells to 900 MHz radiation may alter cytokine expression profile, growth rate, and other functions of the cells. Oxidative stress is another mechanism of action of 900 MHz radiation on whole body and on isolated immune cells. Lu in 2012 claimed reactive oxygen species formation and apoptosis in human blood mononuclear cells induced by 900 MHz mobile phone radiation. Eight hours exposure of peripheral blood mononuclear cells, PBMCs to 900 MHz radiation led to caspase-3 dependent apoptosis of 37% of the cells (Lu et al., 2012). The several published studies by numerous researchers have claimed similar results about 900 MHz radiation induced reactive oxygen species or related markers in different cell types (De Iuliis et al., 2009; Kesari et al., 2011; Maaroufi et al., 2011; Sokolovic et al., 2008). Also, the previous survey by Wellington et al. (2009) revealed that engulfed live _C. albicans_ suppresses the production of reactive oxygen species in phagocytes.

5. Conclusion

This was a preliminary study with meaningful results encouraging us to further investigations. 6 h per day, 7 d per week, over the study period exposure of mice to 900 MHz radiation led to significant retardation of skin wound healing and the survival of exposed mice was notably reduced. The higher yeast load of kidney homogenates in mice exposed to radiation was also noticed. We suppose that radiation induced oxidative stress, disturbance in immune cell functions and altered gene expression may be the possible mechanisms of action of long-term radiation effect on skin wounds and disseminated microorganisms. Further investigations are needed to clarify the importance of the three mentioned mechanisms.

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