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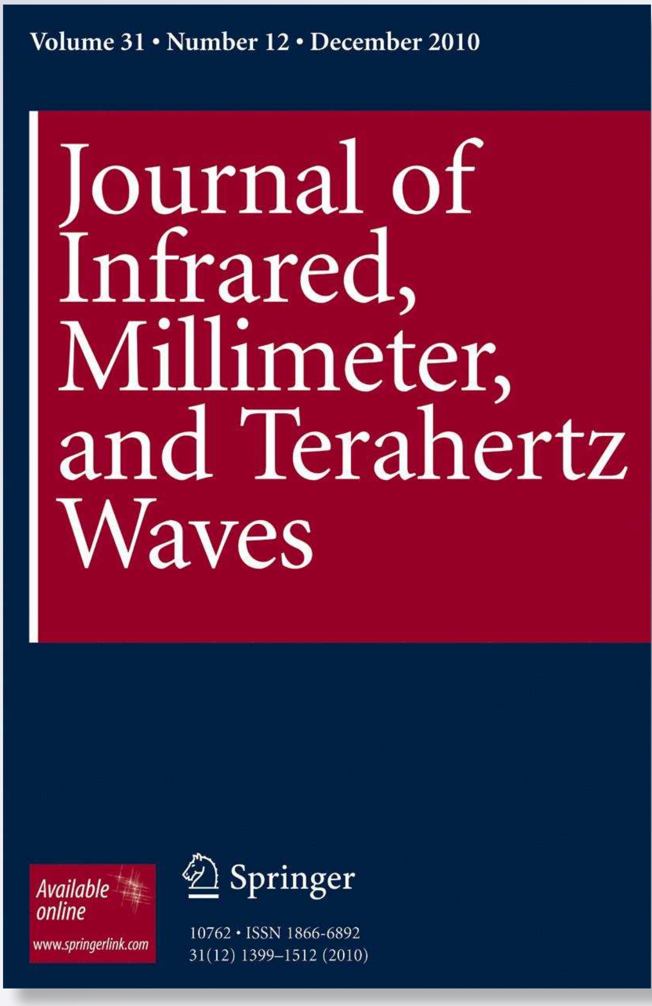

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INVITED REVIEW ARTICLE

Effects of Millimeter Waves Radiation on Cell Membrane - A Brief Review

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Abstract The millimeter waves (MMW) region of the electromagnetic spectrum, extending from 30 to 300 GHz in terms of frequency (corresponding to wavelengths from 10 mm to 1 mm), is officially used in non-invasive complementary medicine in many Eastern European countries against a variety of diseases such gastro duodenal ulcers, cardiovascular disorders, traumatism and tumor. On the other hand, besides technological applications in traffic and military systems, in the near future MMW will also find applications in high resolution and high-speed wireless communication technology. This has led to restoring interest in research on MMW induced biological effects. In this review emphasis has been given to the MMW-induced effects on cell membranes that are considered the major target for the interaction between MMW and biological systems.

Keywords Millimeter waves · Cell membrane · Lipid bilayer · Biological effects

1 Introduction

The millimeter waves (MMW) region of the electromagnetic spectrum, extending from 30 to 300 GHz in terms of frequency (corresponding to wavelengths from 10 mm to 1 mm), is officially used in non-invasive complementary medicine in many Eastern European countries against a variety of diseases such as gastric and duodenal ulcers, coronary artery disease, chronic non–specific pulmonary diseases, traumatism, and tumor [1–8]. Specifically, the most common frequencies used in therapy are 35, 42.2, 53.6, 61.2 and 78 GHz [9]. The millimeter-wave energy of the current medical applications is generally in low power levels below 10 mW/cm^2 producing imperceptible heating of exposed localized areas of skin. For this reason the biological responses might be principally different from those caused by heating. In a few, non-reproducible studies, the effects of MMW often have a sharp,

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resonance-like dependence on the radiation frequency, but they depend relatively little on the radiation intensity [10].

On the other hand, besides technological applications in traffic and military systems, in the near future MMW will also find applications in high resolution and high-speed wireless communication technology [11–13]. This has led to restoring interest in research on MMW induced biological effects.

The study of effects induced by MMW radiation is related to the problem of the interaction between electromagnetic fields (EMFs) and biological systems. This issue is of interest because of fundamental scientific curiosity, potential medical benefits, and possible human health hazards. The latter problem has been studying for decades leading to a big deal of papers indicating potential dangerous effects [14]. The debate is still open, even if several points have been fixed. The action of high-levels fields is well-known and has led to the definition of international safety standards by International Commission of Non-Ionizing Radiation Protection (ICNIRP) in 1998 [14].

2 Biological effects of EM wave: a possible classification

Following classification approaches commonly used in the literature, also if as in every classification some limitations seem inevitable, a first way to classify the biological effects of electromagnetic (EM) wave is related to the energy level of incident radiation. We know that the energy associated with a quantum of electromagnetic radiation can be expressed through the Plank's equation:

$$E = hv$$

This quantity can be or not sufficient to ionize the atoms of the exposed material. In order to structurally modify the biological material the EM wave should transfer an energy quantity notably larger than κT, average kinetic energy of order κT=4.3 10 $^{-21}$ J. For the frequency spectrum we are considering (above 30 GHz) the energy associated with a photon can be considered around 2 10 $^{-23}$ J, at least two order of magnitude less than κT. Further this energy can be considered lower than the activation energy needed for different physic-chemical phenomena (Table 1). Consequently the MMW are classified non-ionizing radiation; they do not destroy inter-atomic bonds, and do not lead to chemical transformations. For these reasons MMW has potential for medical, security and environmental protection applications as mentioned before.

Table 1 Energy activation of some phenomena.

Physic-chemical phenomenon	Energy (eV)	Frequency of EMF (Hz)
atom ionization	≈10	$>10^{15}$
e excitation	1.5-10	$\cong 10^{15}$
covalent bond	5	$\cong 10^{15}$
conformational changes	0.4	$\cong 10^{14}$
hydrogen bond	0.08-2	$\cong 10^{13}$-10^{15}
thermal agitation	0.026	$\cong 5\times 10^{12}$

A second way to classify the biological effects of EM wave is based on the induction of thermal energy into biological system by the electromagnetic energy of incident radiation. We can distinguish between thermal and non-thermal or specific effects. The former are due to a sharp increase in temperature that can be systemic or localized. Such effects typically correspond to high power radiation, greater than 10 mW/cm^2. At the macromolecular level, heating effects will arise from the motion of water molecule dipoles. These effects should be evaluated in order to determine the effective energy absorbed by the target; for such a goal, it could be enough (yet not-trivial) to rigorously characterize the studied system through Maxwell's equations, by applying fundamental EM theorems where the heating process is considered in the energy balancing [15]. In the latter case of non-thermal or specific effects (i.e. effects where temperature plays a non primary role, or it's just one among several physical parameters), mechanisms have not been fully demonstrated, and their comprehension is an existing challenge. Research on specific effects of low-level EM wave has always run into difficulties from the experimental point of view due to the complexity of the biological system involved, the instability of the samples, the weakness of the signal measured and/or the non reproducibility nature of the source. Further research on specific effects of low-level EM wave has attracted few scientists of the Western Countries due to the still open debate on the existence of these specific effects and on their biophysical limits [16].

3 Biological effects of millimeter waves

Several studies on the effects induced by millimeter radiation on biological systems have been reported in the literature. Diverse effects have been observed on cell free systems, cultured cells, isolated organs of animals and humans. The subject has been extensively reviewed by Motzkin [17] and more recently by Pakhomov [3]. At the cellular level these effects are mainly on the membrane process and ion channels, molecular complexes, excitable and other structures. Many of these effects are quite unexpected from a radiation penetrating less than 1 mm into biological tissues [3, 18, 19]. However none of the findings described in the above reviews has been replicated in an independent laboratory, thus they cannot be considered as established biological effects.

Further the interpretation of these reported effects is a matter of controversy, especially regarding whether low intensity MMW exposure (less than 10 mW/cm^2) can produce biological effects via non-thermal mechanism [20]. According to reports that MMW radiation increased or decreased the growth of *E. coli* [21] and yeast [22] depending on different frequencies (136 GHz and 41.68÷41.71 GHz), Fröhlich proposed the existence of electric vibrations in biological systems (coherent oscillations of a section of membrane, proteins, or DNA). He suggested that low power MMW could trigger the excitation of the coherent electric vibrations provided if the biological system is in an active metabolic state [23]. Even if Fröhlich theory has never been confirmed experimentally in reproducible studies, his work suggests that supplying such energy by means of millimeter waves at specific frequency could be a way to regulate the growth of biological entities. As the power densities used in the above cited reports were low (<10 mW/cm^2), the effects on growth rate were considered as non-thermal ones by the reporters. On the other hand, some researchers that attempted to reproduce these non-thermal experiments have reported no effects [24–26], or similar results at higher frequencies (200-350 GHz) [27]. The reasons for these controversial reports could reside in the difference between the biological species, experimental procedures, the uncertainty of biological meaningful exposure metrics, and the difficulties in rigorously performing the required controls.

4 Cell membrane: the major target of MMW interaction

When considering the action of MMW on biological systems the cell membranes are considered the major target for the interaction since they have been theoretically considered to be sensible to coherent excitations above 10^9 Hz [23]. The cell membranes are of immense interest in biological physics: The membrane acts as a gateway to the cell; 30% of all proteins within the body are membrane proteins; and 60% of drug targets are membrane proteins. They control many essential functions in biological tissues. Despite its immense complexity, however, the functionality of the membrane is ultimately determined by its mechanical and electrical properties. In this context the cell membrane is of particular interest because, in principle, interactions between the electrophysiological mechanisms at the cell membrane and electromagnetic fields might be possible [28].

As mentioned before a large number of cellular studies have indicated that MMW may alter structural and functional properties of membranes (Table 2).

Effects of MMW on internal membrane system, i.e. sarcoplasmic reticulum, in skeletal and heart muscles of rat have been reported by Brovkovich *et al*. [29]. The sarcoplasmic reticulum (SR) acts as a calcium source during muscles contraction and a calcium sink during relaxation. The latter is mediated by the transport of calcium into the SR lumen by a Ca-ATPase. The authors measured the rate of Ca^{2+} uptake in SR of skeletal muscle homogenates by an ion-selective electrode in an ATP-containing medium. They indicated that intermittent exposures to continuous wave 61 GHz radiations, at 4 mW/cm^2, significantly activate the Ca^{+2} pump in the SR by 23%. Uninterrupted MMW radiation had no effect in 10 min, but increased Ca^{2+} uptake in SR by 27% in 20 min; and the effect reached a maximum of 48% in 40 min. On the other hand in heart muscle homogenate, even a 5-min exposure enhanced Ca^{2+} uptake in SR by 18%.

It has been reported [30] that exposure to 38-78 GHz radiation, at 5 mW/cm^2, has an indirect frequency-dependent effect on the activity of chloride channels in the cytoplasmic membrane of charophytes (*Nitellopsis obtusa*). Charophytes are water plants useful in physiological studies. These alga cells possess some of the largest single plant cells known, and this makes them relatively easy to manipulate for experimental studies on how cells function. Irradiation for 30-60 min at 41 GHz suppressed the chloride current to zero with no recovery for 10-14 h. Marked inhibitory effects were also found at 50 and 71 GHz, whereas exposures at 49, 70, and 76 GHz enhanced the chloride current up to 200-400%. This activation was reversible, and recovery to the initial value took 30-40 min. MMW induced-heating did not exceed 1°C, and neither activating nor inhibitory effects were related to or could be explained by it. The authors assumed that MMW effect was caused by the modulating effect of radiation on the ATPase activity of chloroplasts, which regulates the Ca^{2+} concentration and pH in the cytoplasm and, thus the activity of chloride channel.

MMW induced-changes on both cooperativity and binding characteristics of the potassium channel activation by internal calcium ions have been reported by Gelyetuk *et al* [31]. The authors measured changes on single Ca^{2+}-activated K^+ channels in cultured kidney cells (*Vero*) by using patch voltage-clamp method (inside-out-mode). The effects of 42.25 GHz (CW), at 0.1 mW/cm^2, were revealed 20-30 min after the onset of the irradiation. The increase or inhibition of the activity of the channels was depending on initial sensitivity of the channels to Ca^{2+} and the Ca^{2+} concentration applied. Successively the authors reported [32] that a 20-30 min preliminary irradiation to 42.25 GHz (CW), at 2 mW/cm^2, of the test solution (100 mM KCl with Ca^{2+} added) would be sufficient to obtain the MMW effects on the channel activity. The possibility of transfer of electromagnetic radiation information through water was attributed to a change in the size

Table 2 Summary of MMW effects induced on membranes. IE = intermittent exposure; I_{AV} = time-averaged incident intensity.

Biological system	End-point / Technique	Frequency / Power	Effects	Reference
Skeletal/heart muscle (rat)	Ca^{2+} pump in sarcoplasmic reticulum / ion-selective electrode	61 GHz 4 mW/cm^2	Increased Ca^{+2} uptake (IE)	Brovkovic [29]
Giant alga cells (*Nitellopsis obtusa)*	Cytoplasmic membrane chloride (Cl^-) channels / voltage clamp	41 GHz 5 mW/cm^2 50, 71 GHz	Cl^- current drops to 0; No recovery for 10-14 h Marked inhibition	Kataev [30]
Idem	Idem	49, 70, 76 GHz 5 mW/cm^2	Cl^- current increased up to 200-400%; Reversible within 30-40 min	Idem
Kidney cell (*Vero*)	Single Ca^{2+} -activated K^+ channels / patch voltage-clamp	42.25 GHz 0.1 mW/ cm^2	Increased activity when < [Ca $^{2+}$] Inhibited activity when > [Ca $^{2+]}$	Geletyuk [31]
Lipid bilayer (BLM) membranes	Proton carriers transport / nonpolarizing Ag/AgCl electrodes	42.25 GHz 3 mW/cm^2	Increased conductance	Cojocaru [34]
Human blood erythrocyte	Energy value of activating Cl^-/OH^- membrane exchange / ionometer hydrogen electrode	38 GHz Max output 0.4 mW	Speeded up transmembrane Cl^-/OH^- antiport	Yemets [38]
Lipid bilayer (BLM) membranes	Gramicidin channel-Capacitance / voltage clamp	53-78 GHz SAR of 2000 W/Kg	Slight decrease Equivalent to heating by 1.1°C	Alekseev [39]
Liposomes	Lipid peroxidation products / chemical analysis	42-64 GHz 0.15 -1 mW/cm^2	20-30% increased peroxides	Andreev [41]
Liposomes (SUV)	$^{\bullet}$OH-dependent lipid peroxidation / chemical analysis	53-61-78 GHz 10 -1- 500 mW/cm^2	None	Logani [42, 43]
Lymnaea neurons	A-type K+ currents and Ca $^{2+}$ currents / Whole-cell voltage-clamp	60-62-75 GHz SAR 0-2400 W/Kg	Thermal	Alekseev [44]
Human peripheral blood and epidermal keratinocyte (HaCaT)	Externalization of phosphatidylserine (PS) / fluorescence – flow cytometry	42.25 GHz 0.55 -1.23 W/cm^2	Ca^{+2} channel then inducing externalization of PS	Szabo [45]

Cationic liposomes loaded carbonic anhydrase	Bilayer permeability / real-time enzymatic kinetics	130 GHz	Increased substrate diffusion across bilayer	Ramundo-Orlando [47]
		I_{AV} up to 17 mW/cm^2		
		2.6-2.7 kV/cm		
Lipid bilayer (Langmuir films)	Lateral pressure dynamics / real-time Wilhelmy technique	60 GHz	Increased lateral pressure	Zhadobov [48]
		0.36 – 0.9 mW/cm^2	No modification lipid domain organization	
Human glial cell line U-251 MG	Endoplasmic reticulum stress / real-time PCR	60.4 GHz	No modification on expression of ER-stress sensor (BiP/GRP78)	Nicolaz [49, 50]
		0.14 mW/cm^2		
Human leukemia K562	Endoplasmic reticulum/TEM analysis	53.37-78.33	Ultrastrucutural modification	Beneduci [51, 52]
		1 μW/cm^2		
Giant vesicles	Morphological alteration / real-time optical microscopy	53.37 GHz	Elongation- induced diffusion of fluorescent dye- increased movement of vesicles- attraction	Ramundo-Orlando [54]
		0.1 mW/cm^2		

and concentration of ice-like clusters consisting of a large number of water molecules bound by hydrogen bonds [32, 33].

The fact that water can be a peculiar kind of information carrier and can have memory of physicochemical and structural properties altered under the action of weak millimeter waves have been also studied by Cojocaru *et al.* [34]. The authors used the same exposure regime as in previous works [32, 33]. The effect of irradiation to 42.25 GHz (CW), at 2 mW/cm^2, directly on water in eliciting the changes of wheat seed germination rate was reported, suggesting that the main acceptor of MMW radiation is water. It's well known that the liquid water is the strongest absorber of millimeter waves: it reduces their energy hundreds to tens of thousands of times [35]. In this context, Ayrapetyan *et al*. [36] reported that the non-thermal effects of irradiation to 160 GHz modulated at 4 Hz, with a specific absorption rate (SAR) of 1.8 mW/g, were directly on water and able to affect heart muscle contractility. The authors suggested that the increase of the specific electrical conductivity of cell bathing water solution during MMW radiation induces possible changes of water dissociation. Furthermore MMW-induced elevation of water dissociations leads to the formation of reactive oxygen species (ROS) that, in turn, modulates Ca-dependent metabolic mechanisms responsible for increased heart muscle contractility [36]. Recently, Kalantaryan *et al*. [37] reported that the effects of MMW irradiation increased the thermo stability and density of water-salt solution of DNA. The authors measured by spectrophotometry and densitometry the changes of the physical properties of DNA solutions irradiated to 64.5 GHz, amplitude modulated at 1 Hz, with incident power density of ≈50 μW/cm^2. It has been suggested that the effect of 64.5 GHz, in correspondence with resonance frequency of oscillations of hexagonal structure of water, was direct on water solution, and by changing the structure of bound water the thermo stability of different nucleotide pairs can consequently change.

Cojocaru *et al*. also reported [34] that irradiation to 42.25 GHz, with energy flux density of 3 mW/cm^2, and the carrier frequency modulated at 16 Hz, increased the electrical conductivity of lipid bilayer membranes. The authors measured the conductance by following the proton transport through black lipid membranes (BLM) in the presence of proton carriers such as bithionol, epigallocatechol and gossypol. The increase of electrical conductivity of lipid bilayer membranes exposed to MMW was depended on concentration of the carriers in the solution, pH of the medium, the dissociation coefficient pK of the carrier, and the ratio between the dissociated forms of the carrier at the membrane boundary (liquid-medium interface). The increase of electrical conductivity of lipid bilayer membranes was related to the enhancement of the convective flow in the aqueous solutions and, especially, in the nonmixed near-membrane layer, which leads to a significant increase in the passive proton transport through membranes.

In this context Yemets [38] reported that exposure to 38 GHz radiation (CW), at a maximum output power of 0.4 mW, lowered the energy value of activating Cl^-/OH^- exchange (antiport of ions), i.e. the energy barrier height, through human erythrocyte membrane. The antiport was realized by placing erythrocytes into non-buffer isotonic medium having lower concentration of chlorine ions. The authors suggested that the MMW radiation indirectly speeds up the transmembrane Cl^-/OH^- antiport, causing motion of the air bubbles in the liquid medium. Their motion provide mixing-up of the liquid medium, which leads to decreasing the thickness of a near-membrane liquid layer, thus facilitating the diffusion of a molecule from intercellular medium into the cell. The mixing-up efficiency is proportional to the temperature thickness gradient formed under MMW irradiation. It is known that when an open surface of the liquid medium is irradiated from above, the latter is heated. Herewith, a temperature thickness gradient is created.

On the other hand, Alekseev and Ziskin [39] reported only slight effects on black lipid membranes (BLM) induced by exposure to 53-78 GHz (CW), at SAR of 2000 W/Kg. MMW exposure decreased reversibly the capacitance of the unmodified BLM by 1.2%± 0.5%. The changes of conductance (ionic channels current) of BLM modified in the presence of gramicidin A, and amphotericin B were small (0.6%±0.4%). At the same time the changes of the transport of lipid-soluble tetraphenylboron (TPhB) anions in BLM modified in the presence of TPhB were reversible increased by 5%±1%. No frequency-specific effects as well as no noticeable structural changes in the membrane were revealed in agreement with results obtained by Motzkin [40], who did not observe any noticeable changes in fluidity of irradiated liposomes. The authors indicated that all changes in membrane capacitance and currents were equivalent to heating by approximately 1.1°C.

It has been suggested by Cojocaru *et al*. [34] that another possible cause of the increase in the electrical conductivity of membranes induced by MMW radiation could be the activation of lipid peroxidation. This assumption was supported by early data [41] on a 20-30% increase of lipid peroxidation products in 15 min after exposure of liposome samples to 42-64 GHz radiations, at 0.15-1 mW/cm^2. The observed effect was attributed to structural changes in membranes and water surrounding the membranes. On the contrary, no enhancement in the formation of lipid peroxides was reported by Logani and Ziskin [42] that exposed liposome samples to frequencies of 53.6, 61.2 and 78.2 GHz (CW), at incident power densities of 10, 1 and 500 mW/cm^2, respectively. These frequencies have been found particularly useful for the treatment of various diseases. Further the same authors [43] indicated that irradiation with 25 mW/cm^2 continuous millimeter waves at 53.6 GHz did not inhibit lipid peroxidation induced by hydroxyl radicals ($^{\bullet}$ OH). Since the power level (25 mW/cm^2) applied in this study is commonly used in cancer therapy, it has been suggested that the beneficial effects of MMW in reducing the toxic effect of chemotherapy could not be related to the inhibition of $^{\bullet}$OH-dependent lipid peroxidation.

Successively, Alekseev and Ziskin [44] reported effects of exposures to 60.22÷62.22 and 75 GHz (CW), with SAR in the range of 0-2400 W/Kg, on *Lymnaea stagnalis* neurons. The authors examined the effects of MMW on Ca^{2+} and fast-inactivating A-type K^+ currents with a whole-cell voltage clamp technique. They concluded that the reversible changes in the amplitudes and kinetics of both currents resulted from the temperature rise produced by irradiation, suggesting that the membrane surface charge and binding of calcium ions to the membrane in the area of channel locations did not change.

Szabo *et al.* [45] studied the effect to 42.25 GHz radiations, at spatial-average incident power of 0.55 and 1.23 W/cm^2, on human peripheral blood and epidermal keratinocyte (HaCaT cells). MMW exposure was capable of inducing reversible externalization of phosphatidylserine (PS) molecules in the membrane of the exposed cells without detectable membrane damage or induction of cell death. Externalization is the rotation of the negatively charged pole of PS molecule from the inner surface of the cell membrane to the outer ones. This phenomenon is considered an early event of programmed cell death allowing recognition of apoptotic cells by phagocytes [46]. Since the presence of extracellular calcium was found to be required for the externalization to occur the authors suggested that the direct effect of MMW was on the calcium channel, indirectly causing externalization of the PS molecule.

Changes on the permeability of lipid bilayer membranes were reported by Ramundo-Orlando *et al*. [47]. The effects induced by 130 GHz radiations, pulse-modulated at low frequencies of 5, 7 or 10 Hz, and at time-averaged incident intensity (I_{AV}) up to 17 mW/cm^2 were studied in real-time during the irradiation of cationic liposomes loaded with carbonic anhydrase. The increase of the substrate diffusion across lipid bilayer induced by MMW was taken as an index of induced permeability change. The higher substrate influx rate resulted at I_{AV} 7.7 mW/cm^2 when the pulse repetition rate was 7 Hz. It is worth nothing that liposome bilayer is about 4 nm thick and that the peak electric field applied through the 130 GHz radiations was up to 2700 V/cm, about 2 orders of magnitude lower than naturally developed across lipid bilayers. Since a clear role of the peak electric field resulted in these studies, with a "window" effect around 2.6-2.7 kV/cm, the authors hypothesized a possible rectification of MMW pulse by liposome, which in turn could lead to change in the lipid membrane permeability.

Zhadobov *et al*. [48] reported of exposures of lipid membrane model to 60 GHz radiations, at 0.36-0.9 mW/cm^2. The authors studied alterations of the lateral pressure dynamics within phospholipid monolayer formed by Langmuir-Blodgett method. The lateral pressure dynamic is a membrane property highly sensitive to small changes in membrane composition, and has a clear mechanistic link to protein function: In the cell membrane the dynamic property of the bilayer can indirectly influence the interaction of membrane protein and bilayer components (lipids or other membrane soluble molecules). The authors reported a significant increase of superficial pressure in phospholipid monolayers during the exposures even at very low power densities (9 μW/cm^2). However no significant transformations in the phospholipid domain organization were observed after 5 h of exposure as resulted by topographic AFM analysis.

Recently, Nicolaz *et al.* [49] reported the absence of direct effect of exposure to 60.4 GHz (CW) radiation. The authors studied the effect of MMW radiations on endoplasmic reticulum (ER) stress in human glial cell line (U-251 MG). ER is a cellular organelle formed of a membrane-interconnected network of tubules, vesicles and cisternae. ER is the site of synthesis and folding of secreted proteins, for this reason is very sensitive to environmental insults and its homeostasis is altered in various pathologies. The authors reported that various time of exposure (24, 46 and 72 h) to 60.4 GHz, at 0.14 mW/cm^2, did not modify the expression of ER-stress sensor (BiP/GRP78) examined by real-time PCR. Successively, the same authors [50] confirmed the previous results on ER stress by using thirteen frequency points between 59.1 and 61.2 GHz (CW) to irradiate the glial cell line in order to evaluate the role of the exact exposure frequency in correspondence of the peak of absorption of molecular oxygen. Great care was also taken to avoid any possible artifactual thermal effects.

On the other hand, Beneduci *et al.* have suggested endoplasmic reticulum to be potential target of MMW. The authors indicated that MMW-irradiated (53.37- 78.33 GHz, 1 μW/cm$^{2)}$ cells line K562 [51] and MCF-7 [52] presented ultrastructural modification of their ER compartment as resulted by transmission electron analysis. Recently, Titushkin *et al*. [53] reported alterations induced by 94 GHz irradiation, at power density of 18.6 kW/cm^2, on Ca^{2+} dynamics in P19-derived neuron-like cells. The authors suggested that the activation of N-type calcium channels and G-protein-coupled receptors in the plasma membrane induced by MMW, in turn could lead to Ca^{2+} release from ER.

Recently, Ramundo-Orlando *et al.* [54] reported the effect of 53.37 GHz radiations on giant vesicles. This membrane model system have a size in the micrometer range (i.e. are of cell size), thus allowing direct observation, under optical microscope, of membrane response to external stimuli. Real time exposures to MMW resulted in three distinct effects: on vesicles geometry, i.e. elongation consisting of an increase in their lengths and changes in their direction angle; induced diffusion of fluorescent dye, di-8-ANEPPS, located in the region between the aqueous phase and hydrocarbon interior of the lipid bilayer; and an increase of movement of vesicles in the aqueous medium and relative attraction among them. A local specific absorption rate (SAR_{avg}) of 0.12±0.01 W/Kg was calculated for the vesicles under irradiation. The authors suggested that the major factor determining the overall perturbation of the vesicles was the action of the field on charged and dipolar residues located at membrane-water interface. It's worth emphasizing that the MMW effects were fully reversible and not dependent on thermal energy.

5 Conclusion

In this review emphasis has been given to the low-level MMW effects on cell membranes. Above all, it should be mentioned that the reported effects are of a non-thermal character, that is, the action of radiation does not produce essential heating of the biological system or destroy its structure. In this context it appears that no permanent structural change of lipid bilayer could arise under low level (less than 10 mW/cm^2) millimeter waves irradiation.

On the other hand, MMW radiation may affect intracellular calcium activities, and, as a consequence, several cellular and molecular processes controlled by Ca^{2+} dynamics themselves. The effects of MMW radiation on ion transport may be the consequence of a direct effect on membrane proteins as well as on phospholipid domain organization. Water molecules seem to play an important role in these biological effects of MMW radiation. Unfortunately, detailed cellular and molecular mechanisms mediating physiological responses to MMW exposure remain largely unknown.

Usually the search at a molecular level is simpler if we can reduce the complexity of our biological samples. This is the case for cell membranes by using model systems. They can be formed by a simple lipid bilayer without interfering components and they give independence from biological activity that can create complication in searching for electromagnetic fields bioeffects. The emphasis is on the search for molecular mechanisms of the membrane effect induced by MMW with different frequencies and power density. Furthermore, replication studies are needed including good temperature control and appropriate internal control samples. It is also advantageous if the future studies are multidisciplinary, invoking an integration of high quality exposure and effects methodologies.

Clearly a significant amount of accurate experimental work is still required in order to fully understand the interactions between MMW radiation and cell membrane.

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