C. R. Physique 14 (2013) 402–411

Contents lists available at SciVerse ScienceDirect

Comptes Rendus Physique

www.sciencedirect.com

Electromagnetic fields: from dosimetry to human health

State of knowledge on biological effects at 40–60 GHz

Le point sur les effets biologiques à 40–60 GHz

Yves Le Dréan [a,*], Yonis Soubere Mahamoud [a], Yann Le Page [a], Denis Habauzit [a], Catherine Le Quément [a], Maxim Zhadobov [b], Ronan Sauleau [b]

[a] *Transcription, Environment and Cancer Group, Institute for Research on Environmental and Occupational Health (IRSET), Inserm U1085, University of Rennes-1, Bat. 13, campus de Beaulieu, 263, avenue du Général-Leclerc, 35042 Rennes cedex, France*

[b] *Institute of Electronics and Telecommunications of Rennes (IETR), UMR CNRS 6164, University of Rennes-1, campus de Beaulieu, 263, avenue du Général-Leclerc, 35042 Rennes cedex, France*

ARTICLE INFO

Article history:
Available online 29 March 2013

Keywords:
Millimetre waves
Biological effect
In vivo and *in vitro* studies

Mots-clés :
Ondes millimétriques
Effets biologiques
Études *in vivo* et *in vitro*

ABSTRACT

Millimetre waves correspond to the range of frequencies located between 30 and 300 GHz. Many applications exist and are emerging in this band, including wireless telecommunications, imaging and monitoring systems. In addition, some of these frequencies are used in therapy in Eastern Europe, suggesting that interactions with the human body are possible. This review aims to summarise current knowledge on interactions between millimetre waves and living matter. Several representative examples from the scientific literature are presented. Then, possible mechanisms of interactions between millimetre waves and biological systems are discussed.

RÉSUMÉ

Les ondes millimétriques correspondent à la gamme des fréquences comprises entre 30 et 300 GHz. De nombreuses applications existent et émergent actuellement dans ce domaine, notamment en télécommunications, imagerie et surveillance. De plus, certaines de ces fréquences sont utilisées en thérapie en Europe de l'Est, ce qui suggère que des interactions avec l'organisme sont possibles. Cette revue vise à résumer l'état des connaissances actuelles sur les interactions ondes millimétriques/matière vivante. Plusieurs exemples représentatifs de la littérature scientifique sont présentés. Enfin, les différents mécanismes potentiellement impliqués dans les effets biologiques des ondes millimétriques seront discutés.

1. Introduction

Due to the increasing demand for mobility and high-data-rate wireless communications, the operating frequencies of emerging civil and military communication systems are shifting towards millimetre waves. The term "millimetre waves" (MMWs) refers to electromagnetic radiations ranging from 30 to 300 GHz in terms of frequency, and from 1 to 10 mm in terms of wavelength in free space. Several sub-bands of the MMW spectrum are particularly attractive for such applications.

* Corresponding author.
E-mail address: yves.le-drean@univ-rennes1.fr (Y. Le Dréan).

1631-0705/$ – see front matter
http://dx.doi.org/10.1016/j.crhy.2013.02.005

Among them, 57–64 GHz frequencies are particularly suitable for short-range secured wireless communications [1], thanks to high level of atmospheric attenuation due to the strong oxygen-induced absorption (16 dB/km).

Compared to other wireless technologies already available at lower microwave frequencies, MMWs offer several advantages, including faster data rates (over 2 Gbit/s), compact size of radiating structures and electronic components, and lower interference between devices. In this context, significant research efforts have been undertaken in the field of MMW technologies – e.g., wireless local area networks (WLAN), wireless personal area networking (WPAN), and more recently body area networks (BAN). Some general public applications have already been introduced to the consumer market, such as Panasonic TVs with 60-GHz wireless platform, and new integrated front-ends at 60 GHz are expected to be commercialised by 2014 on laptops.

MMWs have already been used for numerous applications such as passive imaging for surveillance, active anti-collision automotive radars, security body scanners, radio astronomy or military radars and non-lethal weapons. Moreover, MMWs have been used for therapy in some Eastern European countries. MMWs alone, or in combination with other treatment, gave promising clinical results in the cure of various diseases, including ulcers, pain relief, cardiovascular diseases, wound healing, bronchial asthma, skin disorders or cancers [2]. This is strong evidence suggesting that under certain conditions, MMWs can interfere with human physiology.

To determine whether the biological effects observed under therapeutic conditions can also appear in other exposure scenarios, including exposure to wireless communication signals, is a major environmental, economical and social issue. In this framework, a better knowledge of the potential influence of MMWs on the biological systems, and particularly on the human body, is of uppermost importance.

2. Potential biological effects of MMWs: thermal *vs* non-thermal effects

MMWs are non-ionising radiations as the photon energy (10^{-4} to 10^{-3} eV) remains several orders of magnitude below the level required to ionise biological molecules (typically > 10 eV). This is important because non-ionising and ionising radiations generate fundamentally different effects in living organisms. Moreover, energy associated with MMWs is not sufficient to break ionic, hydrogen or van der Waals bonds, which are involved in interaction between biological molecules within cells. However, MMWs can induce rotation of some free molecules with a dipole moment. Indeed, these spectral signatures are used in spectrochemistry to identify diatomic and triatomic molecular groups. The consequence of all this molecular agitation is that heating is the major effect resulting from the absorption of the MMW energy. Because of this well established heating effect, there is no longer any doubt that MMW radiations can affect biological functions. Currently, one of the open questions is the following: where is the threshold in terms of the exposure conditions (power, frequency, duration, etc.) between safe exposures and potentially hazardous exposures?

Thermal effects appear after exposure to incident power density (IPD) above 5–10 mW/cm^2. High-intensity MMWs act on human skin and cornea in a dose-dependent manner: heating sensation may occur at low-power densities, then followed by pain at higher exposures, and even by physical damage at very high powers. The active denial systems rely on this thermal effect. People exposed to high-power 94-GHz radiations undergo a sudden increase in their superficial temperature [3], resulting in a quick burning sensation and in an escape reaction from the MMW beam. It was demonstrated on human volunteers that the pain sensation was correlated with an increase in surface temperature [4]. In this review article, we will not deal with high-power exposures leading to an excessive hyperthermia, because most of new civil wireless MMW applications will operate at power density levels low enough to not induce acute thermal effects.

Nevertheless, slight or moderate elevations of temperature are major issues for anyone interested in the biological effects of MMWs. The existence of pure electromagnetic bio-effects, strictly independent of temperature rise, is still controversial. This can be explained by the fact that, experimentally, this task is awkward. Unlike toxicologists who can demonstrate the deleterious effects of chemicals by using high levels of exposure, experimenters cannot use high exposure levels without trigger hyperthermia. This point is particularly important when using MMWs, because the energy is very locally absorbed by the body surface, which generates significantly higher specific absorption rates (SAR) compared to those obtained at lower microwave frequencies for identical power density values. Temperature elevation causes several effects at the cellular level. It can impact cell growth, cell morphology and cell metabolism. It can also induce the production of reactive oxygen species and increase DNA, lipid, and protein damages. All these parameters have often been highlighted in studies assessing the biological effects of electromagnetic fields. Unlike temperatures above 45 °C, temperatures ranging between 40 and 44 °C are not lethal to most mammalian cells, but such exposures activate an adaptive mechanism called heat-shock response (HSR). During HSR, cells synthesise many proteins involved in protection and cell survival. Among these proteins, the heat-shock protein 70 (HSP70) is dramatically over-expressed (Fig. 1A). This factor is therefore used as a convenient biomarker to monitor the impact of environmental factors on cells. Because acute HSR is not observed below 39 °C, the scientific community often considers that moderate elevation of temperature ($\Delta T < 2$ °C) has no significant consequence for the cell. However, exposure duration may be a critical parameter. Fig. 1B clearly shows that prolonged exposure to 38 °C induces a weak HSP70 overexpression by a factor 1.5 to 2.5. Temporal expression profile indicates a peak for a 2-h exposure, suggesting a dynamic response to transiently adjust the intracellular level of HSP70. So, minor temperature changes with no deleterious effect may induce subtle cellular modifications. Therefore, appropriate dosimetry and temperature control are essential to determine the existence of non-thermal effects of MMWs.

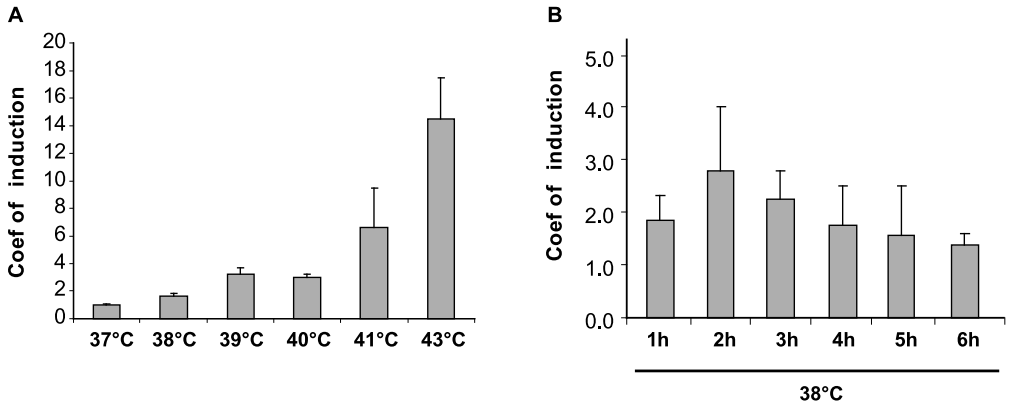

Fig. 1. Representative heat-shock response in human keratinocyte cell line (HaCaT). (A) Overexpression of HSP70 mRNA in response to acute heat shock (45 min at temperature indicated below histograms). (B) Levels of HSP70 mRNA after moderate heat shock (38 °C for the duration indicated below histograms).

Although the biological effects of low-intensity MMWs have been studied for decades, particularly in Eastern European countries, very little reliable and reproducible data are available. In 1998, Pakhomov and colleagues [5] have written a complete review on MMW biological effects. Thus in this review, we focus on the more recent studies published during this last decade. The MMW-induced biological effects are related to several factors: 1) the frequency, 2) the power density, 3) the duration of exposure, and 4) the tissue or cell type. This could explain why the reported biological effects are often inconsistent: depending on the biological model and the exposure conditions, many parameters differ from one study to another. Moreover, the biological function tested *in vivo* or *in vitro* may itself be highly dependent upon uncontrollable biological and environmental factors. All these factors may explain the heterogeneity of the published data.

It is important to note that the strongest argument in favour of an MMW biological effect comes from the use of these radiations in therapy. However, in therapeutic modality, the applied IPD used generates a slight and local increase of temperature (up to 1–2 °C), and consequently it cannot be classified as purely non-thermal. So, according to a limited number of scientific publications, there is no sufficient evidence that non-thermal effects of MMW exposure exist. Current exposure guidelines [6] are essentially established to avoid thermal hazards by applying a certain safety margin for the general public and workers. For far-field exposures (averaged over 20 cm^2) in the 60-GHz band, the IPD is limited to 1 mW/cm^2 for the general public and to 5 mW/cm^2 for workers. However, higher IPD is allowed for IPD averaged over 1 cm^2 (20 mW/cm^2 for general public and 100 mW/cm^2 for workers). Such exposure conditions may correspond in practice to some near-field scenarios [7,8], as expected in future MMW body-centric communication systems in which antennas will be placed directly on the body or integrated into garments [9]. It can be assumed that exposure to 20 mW/cm^2 will result in a very local heat-shock response. However, as the exposed area is very limited, the heat dissipation is efficient and therefore, no thermal damage to tissues is expected.

Another important property of MMWs is their shallow penetration depth into biological tissues and solutions [10]. It is of the order of a few tenths of millimetres to several millimetres, depending on frequency and tissues, indicating that the skin or near-surface zones of the tissues are the main targets for MMW radiations. One may wonder how radiations with such a shallow penetration into the body can have biological effects at the level of the whole organism. However, it should be noted that skin is not isolated from the rest of the body, and this organ contains capillaries and nerve endings. So, it is possible that MMW bio-effects could be transmitted through secreted molecular factors by the skin or through the nervous system [11,12]. Three frequencies are commonly employed in therapy: 42.2, 53.6, and 61.2 GHz. The choice of these frequencies was empirical, but it is possible that the penetration depth of these waves is involved. This allows us to target a precise subpopulation of cells in the skin (Fig. 2). How MMWs can efficiently act on so many different diseases is still largely unknown. Nevertheless, some hypotheses have been explored in recent years.

3. Effect of millimetre waves on gene expression

Effect of MMW exposure on gene expression is an important topic, but, curiously, only a limited number of studies have focused on it. Moreover, most of them were restricted to cellular stress biomarkers expression. Change in gene expression is quantitatively correlated with an adaptive cellular behaviour. So, its study may facilitate our ability to understand how cells sense and react to MMW exposures.

According to the literature, DNA and proteins are the living cell components the most affected by physical and chemical conditions. However, these two categories of molecules have differential vulnerability to environmental stresses. Conditions affecting DNA, the so-called “genotoxic stresses”, include, for example, ultraviolet light, ultrasound and ionising radiations, to which proteins are particularly resistant, while proteins are very sensitive to other physical conditions, such as hyperthermia and to certain chemical components, such as heavy metals. In fact, DNA damages seem to be induced by higher

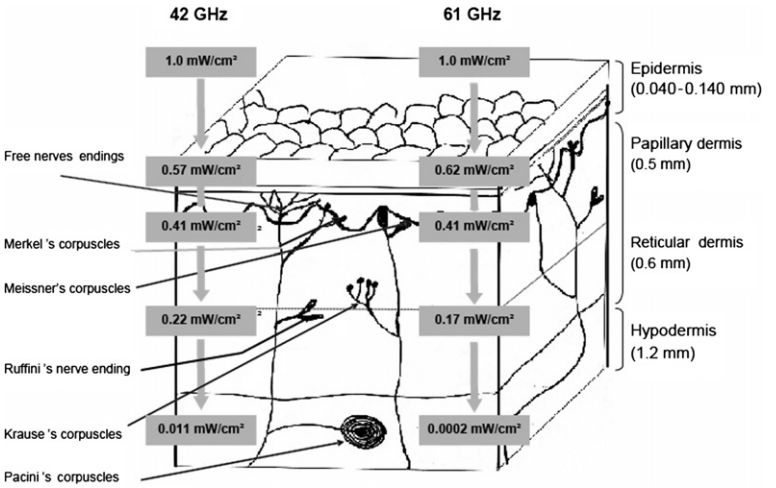

Fig. 2. Schematic representation of the skin's structure and penetration depth of MMWs.

energy treatments, and it was published that MMWs are not genotoxic [13]. Contrary to DNA, whose structure is relatively simple and whose principal deterioration consists of breaks of covalent bonds, the structure and solubility of proteins are conditioned by complex three-dimensional folding up, particularly fragile and affected by relatively weak amounts of disruptive treatments. Protein misfolding is very harmful for cells since it is inherently a self-propagating process, recruiting surrounding proteins by hydrophobic contacts, which may lead to deleterious protein aggregation. Consistently, cells have developed sophisticated protein-quality-control machineries (e.g., HSP and chaperone proteins), whose expression is highly reactive to proteotoxic environment. A scanty number of studies point out the absence of HSP overexpression after exposure to low-power MMWs. It was demonstrated that, if care is taken to avoid thermal effects, no notable change in chaperone expression is detected [14,15]. The cellular responses to proteotoxic stresses are strikingly dependent on the primary location of denatured proteins. The case of protein insults within the secretory pathway is perhaps the most sophisticated and sensitive system. As much as one third of the cellular proteins are confined in the endoplasmic reticulum (ER). Therefore, this cellular organelle is very concentrated in proteins and any disruption of its homeostasis has dramatic consequences. In order to further explore the potential effect of MMWs, we analysed the effect of these radiations on the reticulum stress. ER is very sensitive to environmental insults and disruption of its function has been found in many diseases. Our results clearly demonstrate that low-power MMWs (0.14 mW/cm^2) within the 59–61.2 GHz frequency range does not trigger ER stress [16,17]. All together, these data indicate that low-power MMW exposures do not induce acute proteotoxic stresses that necessitate a transcriptional response.

Therefore, it is crucial to consider other cellular targets to further analyse the effect of MMW radiations. By profiling the global RNA content, it is possible to monitor in parallel all expressed genes to assess the global cellular response to MMWs. For this purpose, DNA microarray is the most powerful tool. In 2008, a study has investigated the alterations in gene expression profile and histology of rat skin after exposure to 35-GHz radiations [18]. Skin samples were collected at 6 h or 24 h after exposure. Using Affymetrix GeneChip technology, the authors found changes in more than 50 genes in MMW-exposed rats compared to shams (Fig. 3A). Most of them were associated with proteotoxic and oxidative stresses, immune response, and tissue matrix turnover. However, as the IPD used in this study was high (75 mW/cm^2), it is very likely that the effects were due to an increase in temperature. The skin first reacted to the acute heat shock (functional group of genes mainly found after 6 h), then skin injury led to subsequent recovery, involving inflammation and tissue repair processes (functional group of genes mainly found after 24 h).

Using DNA microarrays (44K-Agilent), our group investigates the potential response of human keratinocytes exposed to low-power MMWs [19]. Under these conditions (60 GHz; 1.8 mW/cm^2), the measured maximal temperature increment was around 0.8–0.9 °C, which does not trigger massive overexpression of HSP. Two statistical analyses were performed. First, using a stringent test with a Benjamini–Hochberg correction, no significant difference in gene expression was observed. Secondly, a less stringent t-test was employed, and 130 transcripts were found to be potentially modulated after exposure (Fig. 3B). Most of the responsive genes were found after a 6-h exposure, suggesting a transient and reversible cellular response. This might reflect the temporal dynamic of the cell adaptation, including early transcriptional adjustment and rapid return to equilibrium. PCR validation showed that at least 80% of the 130 identified genes are false positives, because they were selected after removing the statistical corrections. Five genes out of the 24 tested were confirmed by RT-PCR to be really differentially expressed after a 6-h exposure (i.e. CRIP2, PLXND1, PTX3, SERPINF1, and TRPV2). After a 24-h exposure, only one gene was confirmed as differentially expressed (TRPV2). We can therefore conclude that keratinocytes are very little affected by short-term low-power MMW exposure.

In order to quantify potential cellular response induced by higher-power MMW exposures, we modified our exposure system. Under near-field conditions, we were able to increase the power density up to the IPD used in therapy (several tens

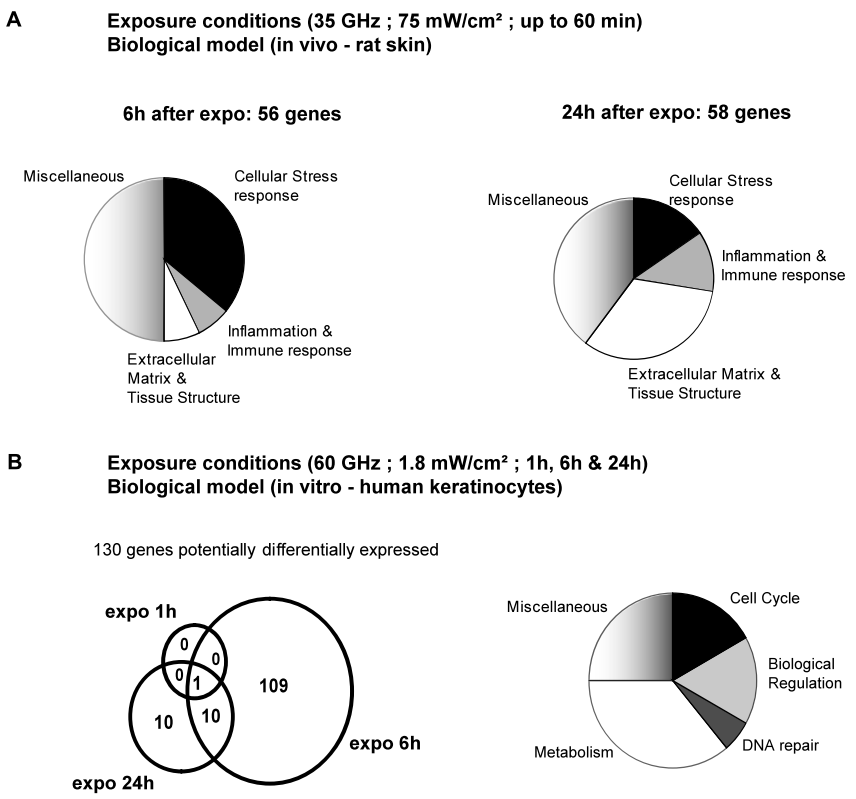

Fig. 3. Repartition of genes entities found differentially expressed after MMW exposure. (A) High-power MMWs generating thermal effects. Gene distribution according to their biological functions [18]. (B) Low-power MMW exposure. Venn diagrams of overlapping gene expression patterns of the 130 potential differentially expressed genes across the three time points. Gene distribution, according to their biological functions [19].

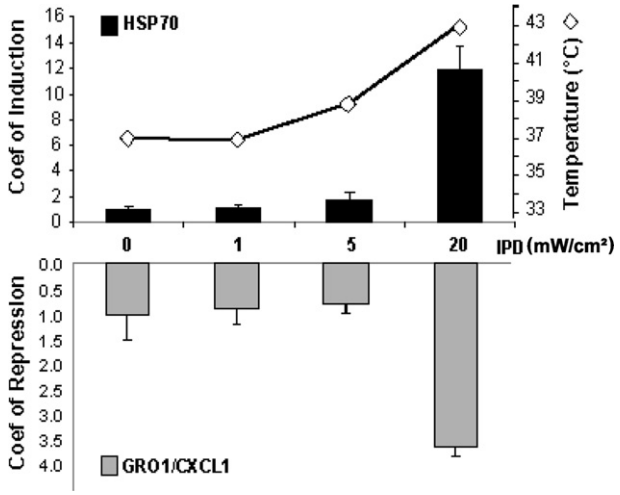

Fig. 4. Transcriptional change of sensor genes after a 24-h exposure at different IPD. Diamond curve shows steady-state temperature in the culture medium, during exposure. mRNA levels were quantified by real-time PCR (black histograms: HSP70; grey histograms: GRO1/CXCL1).

of mW/cm^2). Then, by real-time PCR, we measured the basal expression levels of two sensor genes: HSP70, which is over-expressed in response to temperature rise, and GRO1/CXCL1, a gene involved in inflammatory processes which was found to be modulated by high-power MMW exposure [19]. A temperature control experiment was also carried out. As presented in Fig. 4, repression of GRO1/CXCL1 expression was observed at 20 mW/cm^2 (averaged over the cell layer IPD), a condition that causes a temperature rise sufficient enough to induce a heat-shock response.

The results obtained by our group clearly show that increase of IPD and its associated temperature rise are the main parameters affecting cells. Under athermal conditions, the keratinocytes do not dramatically change their gene expression program. However, our experiments have also highlighted the fact that keratinocytes are particularly resistant to diverse

environmental stresses. So, it is possible that these cells, although being the most abundant ones in the skin, are not the most sensitive to MMW exposure and associated local temperature increases. Timing and duration of exposure should also be taken into account. It is possible that changes in gene expression result in a long process, and that only chronic exposure may permit to observe them. Our data suggest that low-power MMW exposure does not instantly activate transcription factors or stress-dependent signalling pathways, but we cannot rule out the existence of more subtle changes. For example, it is well known that environmental conditions (e.g., pollutants) may exert their long-term effects through epigenetic modifications. The epigenetic programs govern chromatin structure and remodelling, which permits a fine tuning of gene expression. The field of environmental epigenetics is still nascent, and no one can exclude an impact of exposure to electromagnetic waves on epigenetic regulation.

4. Effect of millimetre waves on cell proliferation and differentiation

Medical application of MMWs began in Eastern European countries in the 1970s. MMWs have been used for a large spectrum of diseases, including cancer. However, only few groups in the world conducted well-controlled studies with an appropriate dosimetry on proliferative or cellular differentiation effects linked to MMW exposure. Moreover, it is very difficult to draw clear conclusions from those studies, first because of contradictory observations, and second because of the heterogeneity of models and exposure parameters.

First, several studies have provided data on direct effect of MMWs on *in vitro* cell cultures. An Italian group described an antiproliferative effect of 53–80 GHz irradiation under athermal conditions on different cell lines. In a first paper, they observed an inhibition of cell growth and morphological alterations of the RPMI 7932 human melanoma cell line in response to low-power MMW exposure [20]. Similar results were described in a second paper with the MCF-7 human breast cancer cell line [21]. It is interesting to note that the cells were irradiated directly on the open surface of a cell culture dish, while it is well known that, in this range of frequencies, the penetration depth in the culture medium is less than 1 mm. A third paper, using exposure from underneath, confirmed these results in the K562 human erythromyeloid leukaemia cell line and described an enhancement of glucose metabolism associated with an increasing number of mitochondria [22]. Finally, in a last paper [23], the same group observed that 42.20- and 53.57-GHz exposures do not affect the proliferation rate or the cell cycle of RPMI 7332 cells. In China, the group of Guan Gwen Wu uses a cultured mesenchymal stem cells (MSCs) model to study 30–40 GHz MMW athermal exposures. Under their experimental conditions, MMW radiations seem able to induce MSCs differentiation into chondrocytes [24]. In addition, they also observed that MMW exposure prevents apoptosis induced by sodium nitroprussiate treatment and reduces caspase-3 activity [25]. Moreover, in two other papers, the same group observed a decrease in number of G0/G1 and G2/M chondrocytes in response to MMW exposure and an increase of S phase cells [26,27]. They concluded that MMW exposure seemed to induce chondrocyte differentiation and proliferation, and they speculated that MMWs can inhibit osteoarthritis or natural apoptosis of chondrocytes. These results were partially confirmed by another Chinese group from the same university working on a rabbit surgically-induced model of knee osteoarthritis exposed to 37.5-GHz MMWs [28]. More recently, the group of Guan Gwen Wu described successively an antiproliferative effect of MMWs through apoptosis induction in SW1353 cells [29], and confirmed a proliferative effect through inhibition of mitochondrion-dependent apoptosis pathway in chondrocytes [30]. It appears very difficult to reconcile such disparate data. Altogether, these results are inconsistent because opposite effects are sometimes observed. Depending on studies, MMWs seem to induce or inhibit apoptosis, promote or not cell cycle progression, enhance or suppress cell proliferation, or simply do not lead to any modifications at the cellular level. However, the large variety of cell types and exposure protocols might explain this heterogeneity. Additional studies will be necessary to conciliate such heterogeneous results on cell proliferation/differentiation, and probably the answer will come from studying all potential indirect effects.

The effect of MMWs on cell proliferation was also analysed via *in vivo* models. The group of Behari from India worked on the effects of chronic exposure at 50 GHz on the reproductive system of the rat [31]. In sperm, they observed an increase in the percentage of apoptosis and a significant decrease in the S phase and G2/M transition phases of cell cycle in the exposed group, as compared to the sham control. Their results are surprising because the authors performed their analysis on mature spermatozoids that are not supposed to divide anymore. However, their results globally point out toward an induction of oxidative damage to cells and an enhancement of the production of reactive oxygen species (ROS) in response to MMW exposure. This induces a genotoxic stress that is observed by the authors, but in contradiction with previous publications [13]. Since 2003, the Ziskin group at Temple University has published a long series of papers reporting, in a mice model, the potential interference of MMW exposure with cyclophosphamide (CPA), a drug commonly used in the treatment of the human malignancy. They found that a 61.22-GHz MMW exposure is able to suppress subcutaneous tumour growth [11]. In 2006, they reported that MMWs at the same frequency lead to a reduction of CPA-induced metastases and an enhancement of natural killer cell activity [32]. Thus, it is possible that the beneficial effects of MMWs in the case of cancer treatments are due to indirect effects. MMWs could stimulate the immune system, which in turn would remove the tumour via mechanisms already well known and used in immunotherapy.

5. Effect of millimetre waves on immune and inflammatory systems

During the last decade, the effects of MMWs on the immune system have been extensively studied, showing that they can modulate immune responses. For example, it was shown that MMWs increased the phagocytic activity of

macrophages, enhanced T-cells proliferation, normalised the ratio of CD4+/CD8+ T-lymphocytes, and increased the amount of B-lymphocytes [2].

Makar and collaborators demonstrated in mice irradiated at 61.32 GHz (31 mW/cm^2) that MMWs can restore tumour necrosis factor-alpha (TNF-α) production, which had been inhibited by an anti-cancer drug treatment [33]. This TNF-α production by peritoneal macrophages was accompanied by a significant increase in interferon-gamma (IFN-γ) release by splenocytes and enhanced proliferative activity of T-cells [34]. However, it is well known that IFN-γ, synthesised by lymphocytes, markedly increases TNF-α production in monocytes and macrophages. The same authors have also shown that 42.2-GHz MMWs at 31 mW/cm^2 can up-regulate natural killer (NK) cell functions [35]. In conclusion, we can assume that MMWs enhance proliferative activity of T-cells and stimulate inflammatory response through TNF-α and IFN-γ up-regulation.

Low-power 42-GHz MMWs at 0.1 mW/cm^2 have also been described as having anti-inflammatory actions [36,37]. Using a model of local acute inflammation in mice, Gapeyev et al. have shown that MMW exposure reduces both the footpad oedema and local hyperthermia induced by zymosan [38]. These anti-inflammatory effects seem to be strongly dependent on the frequency, intensity, and duration of the exposure [38,39]. Moreover, the magnitude and kinetics of the effects of MMW treatments were comparable with those induced by therapeutic doses of the well-known anti-inflammatory drug: Diclofenac, a cyclo-oxygenase inhibitor. Furthermore, the combination of those two treatments (MMWs and drugs) caused a partial additive effect of decrease in the footpad oedema [38]. Using a rat model, it was shown that plasma from MMW-exposed animals, compared to sham-exposed, was able to activate cultured macrophages, which led to an increasing expression of 11 proteins [40]. All these proteins are associated with inflammation and oxidative stress. However, it should be noted that similar results were obtained with plasma from heat-exposed animals. In summary, the data presented here indicate that environmental heat and MMWs may cause a release of active mediators into the systemic circulation.

One of the major scientific challenges is to understand the cellular and molecular mechanisms that are responsible for these effects. With a low penetration in the body (less than 1 mm), the main target of MMWs are epidermis and dermis that are composed of keratinocyte cells, Langerhans cells, blood vessels, and nerve endings. Several studies have been performed to determine whether MMWs could directly activate skin cells. One can presume that these cells could secrete some factors in the general blood circulation, which will modulate the activity of the immune and inflammatory systems. A large-scale study on gene expression in skin rat after high-power MMW exposure [18] found an increase in CXCL-1, CCL-2 and S100a9 expression. These chemokines are involved in neutrophils and macrophages migration. Other studies, performed on an *in vitro* model, described limited effects of MMWs on keratinocytes. A very modest increase in the intracellular level of IL-1β was found, while no changes in the expression of other chemotaxis factors (IL-8, RANTES and IP-10) were detected [14,41]. Moreover, our group, using microarray screening, did not find any major change in gene expression of factors involved in immune system after acute 60-GHz exposure of human keratinocytes [19]. Altogether, these data suggest that low-intensity MMWs do not directly activate keratinocytes into secreting signalling factors.

As very few secreted factors were up to now identified in skin and particularly in keratinocytes, a new hypothesis has been formulated. Activation of immune system by MMW exposure could be indirect through stimulation of the peripheral neural system. This hypothesis was first described by Makar and collaborators [34]. Biological effects of MMWs might be initiated by activation of free nerve endings in skin. Then, production of endogenous opioids may up-regulate the release of immunostimulatory cytokines from T-cells and macrophages (INF-γ and TNF-α). More recently, the link between endogenous opioids activation and immunomodulation effects of MMWs was confirmed [42].

6. Effect of millimetre waves on peripheral and central nervous systems

As mentioned previously, MMWs have been used for several medical applications notably pain release, such as treatment of headache, joint pain, postoperative pain, and painful diabetic neuropathy [43]. MMWs are applied for 15–30 min and experimental data showed that optimal effects were obtained with a frequency of 61.22 GHz [12]. The hypoalgesic effect is dependent on the power density applied and is not observed with an IPD lower than 0.5 mW/cm^2 [44]. Therefore, IPDs between 5 and 15 mW/cm^2 are usually used for hypoalgesic effects. This power level produces a slight temperature increase, but the patient perceives neither pain nor heat [45]. As skin exposure is very local during this MMW pain therapy, the most intriguing question is the evaluation of the role of the exposure site. Hypoalgesic effects were found in animal and human with MMW exposures to acupuncture points, or by exposing skin areas with high nerve endings concentration [46–49]. These data point out that the peripheral neural system is the link between MMWs and pain treatment. This was confirmed by experiments in which nerve transections were performed in rats [50]. This surgical operation was used to isolate the MMW-exposed area to the brain. In that case, the hypoalgesic effect of MMWs was completely abolished, demonstrating that a neural connection between skin and central nervous system is necessary. Later, it was confirmed that the hypoalgesic effect is mediated by the central nervous system, particularly by the hypothalamic area. Pre-treatment with specific opioid antagonists completely blocked the MMW effect on pain release [51]. This data suggests that MMWs act through a release of endogenous opioids, which are natural molecules involved in pain tolerance. In accordance with this hypothesis, it was found that MMW exposure significantly increased the enkephalin (a natural opioid peptide) level in the midbrain and the hypothalamic areas [12].

The precise mechanisms involved in the effect of MMWs on pain suppression are still unknown. We still do not know which nerves endings or sensory receptors are targeted by MMWs (Fig. 2). However, several publications have highlighted

that MMW exposure can directly impact nerve activity. For example, it was found that MMWs (42.25 GHz) could induce changes in the electrical activity of the murine sural nerve [52]. However, the IPDs required for observing this effect are much higher (45 mW/cm^2 and 160 mW/cm^2) than those used for therapeutic purposes. It is interesting to note that the authors did not detect changes in the firing rate for exposure with lower intensities (10–30 mW/cm^2). By contrast, Siegel and Pikov from the Jet Propulsion Laboratory (CA, USA) were able to observe changes in the firing rate of neurons at power levels as low as 0.3 μW/cm^2 [53].

At the cellular level, two main phenomena on cell permeability could be considered to explain nerve ending activation. The first one is the protein channels included within membranes and the second one is the phospholipid bilayer by itself. Works on neuronal firing suggested that MMW effect is independent of Ca^{2+} pumps and impacts directly the plasma membrane, either by modifying the activity of other ion channels or by directly modifying the phospholipid bilayer [54]. It is important to note that MMWs were already found to modify the plasma membrane bilayer structure. First, high-power MMW exposure induces a transient externalisation of phosphatidylserine (PS), a lipid normally located in inner-leaflet of cell membranes [55]. Generally, PS externalisation is associated with cell death, but curiously, the externalisation induced by MMW exposure was reversible and not associated with cellular damages. Secondly, our group evidenced that MMW exposure to 60 GHz (0.9 mW/cm^2) reversibly increased the lateral pressure on artificial biomimetic membranes [56]. Thirdly, it was also demonstrated that exposure to 53.3 GHz or 130 GHz modifies both the shape and permeability of liposomal vesicles [57,58]. The exact mechanisms of MMW action on membranes or biological systems remain unknown. One can imagine that the MMW radiation can interfere with the orientation of charged and dipolar molecules, which can lead to changes at the membrane–water interface. If such a change modifies the neural membrane's permeability, it could contribute to stimulate neuron endings or affect the electric signal transmitted to the rest of the body, then modify environmental perception, including pain sensation.

7. Conclusions

A comprehensive analysis of *in vitro* and *in vivo* studies shows that the majority of the biological effects have been observed for MMW exposures with IPD above 5 mW/cm^2. At these power levels, exposure causes a local heating, which means that potential pure electromagnetic effects are difficult to delineate from the thermal ones. For lower IPD levels, which do not induce thermal effects, the data are more controversial. Often, the models and the exposure parameters are quite different, and it is not easy to harmonise and to reconcile such disparate experiments and results. Among the described biological effects, those showing hypoalgesic effects are probably the more reliable, as positive data, using blind tests with animals or human volunteers, have been published by different laboratories.

Underlying mechanisms are still unknown. At the cellular level, it stands out from the literature that skin nerve endings are probably the main targets of MMWs and the possible starting point of numerous biological effects. At the molecular level, there is no acceptable theoretical model, except for the rotational dispersion of free water molecules. The energy of MMW radiation is too low to directly disrupt any biochemical interaction, such as van der Waals or hydrogen bonds. Only a resonance-type interaction might lead to an appreciable biological effect. However, the existence of such a resonance at the cellular level is still unknown. According to the literature, cell membranes represent a promising potential target and this topic requires further experimental investigations in the coming years.

Finally, well-controlled and reproducible studies with an appropriate dosimetry are still needed to well characterise and quantify the biological effects of MMWs and their thresholds.

Acknowledgements

The authors want to thank the French National Research Agency (ANR), and the French Agency for Food, Environmental and Occupational Heath & Safety (ANSES) for their support.

References

[1] R.C. Daniels, J.N. Murdock, T.S. Rappaport, R.W. Heath, 60 GHz wireless: up close and personal, IEEE Microw. Mag. 11 (2010) 44–50.
[2] M.A. Rojavin, M.C. Ziskin, Medical application of millimetre waves, QJM 91 (1998) 57–66.
[3] J.C. Debouzy, D. Crouzier, V. Dabouis, R. Malabiau, C. Bachelet, A. Perrin, Biologic effects of millimeteric waves (94 GHz). Are there long term consequences? Pathol. Biol. 55 (2007) 246–255.
[4] T.J. Walters, D.W. Blick, L.R. Johnson, E.R. Adair, K.R. Foster, Heating and pain sensation produced in human skin by millimeter waves: comparison to a simple thermal model, Health Phys. 78 (2000) 259–267.
[5] A.G. Pakhomov, Y. Akyel, O.N. Pakhomova, B.E. Stuck, M.R. Murphy, Current state and implications of research on biological effects of millimeter waves: a review of the literature, Bioelectromagnetics 19 (1998) 393–413.
[6] ICNIRP, Guidelines for limiting exposure to time varying electric, magnetic, and electromagnetic fields (up to 300 GHz), Health Phys. 74 (1998) 494–522.
[7] M. Zhadobov, R. Sauleau, R. Augustine, C. Le Quément, Y. Le Dréan, D. Thouroude, Near-field dosimetry for in vitro exposure of human cells at 60 GHz, Bioelectromagnetics 33 (2012) 55–64.
[8] N. Chahat, M. Zhadobov, L. Le Coq, S.I. Alekseev, R. Sauleau, Characterization of the interactions between a 60-GHz antenna and the human body in an off-body scenario, IEEE Trans. Antennas Propagat. 60 (2012) 5958–5965.
[9] N. Chahat, M. Zhadobov, L. Le Coq, R. Sauleau, Wearable end-fire textile antenna for on-body communications at 60 GHz, IEEE Antennas Wirel. Propag. Lett. 11 (2012) 799–802.

[10] M. Zhadobov, N. Chahat, R. Sauleau, C. Le Quément, Y. Le Dréan, Millimeter-wave interactions with the human body: state of knowledge and recent advances, Int. J. Microw. Wireless Technol. 3 (2011) 237–247.
[11] A.A. Radzievsky, O.V. Gordiienko, I. Szabo, S.I. Alekseev, M.C. Ziskin, Millimeter wave-induced suppression of B16 F10 melanoma growth in mice: involvement of endogenous opioids, Bioelectromagnetics 25 (2004) 466–473.
[12] A.A. Radzievsky, O.V. Gordiienko, S.I. Alekseev, I. Szabo, A. Cowan, M.C. Ziskin, Electromagnetic millimeter wave induced hypoalgesia: frequency dependence and involvement of endogenous opioids, Bioelectromagnetics 29 (2008) 284–295.
[13] Vijayalaxmi, M.K. Logani, A. Bhanushali, M.C. Ziskin, T.J. Prihoda, Micronuclei in peripheral blood and bone marrow cells of mice exposed to 42 GHz electromagnetic millimeter waves, Radiat. Res. 161 (2004) 341–345.
[14] I. Szabo, M.R. Manning, A.A. Radzievsky, M.A. Wetzel, T.J. Rogers, M.C. Ziskin, Low power millimeter wave irradiation exerts no harmful effect on human keratinocytes in vitro, Bioelectromagnetics 24 (2003) 165–173.
[15] M. Zhadobov, L. Debure, R. Sauleau, L. Le Coq, D. Thouroude, D. Michel, Y. Le Dréan, Millimeter wave radiations at 60 GHz do not modify stress-sensitive gene expression of chaperone proteins, Bioelectromagnetics 28 (2007) 188–196.
[16] C. Nicolas Nicolaz, M. Zhadobov, F. Desmots, R. Sauleau, D. Thouroude, D. Michel, Y. Le Dréan, Absence of direct effect of low-power millimeter-wave radiation at 60.4 GHz on endoplasmic reticulum stress, Cell Biol. Toxicol. 25 (2009) 471–478.
[17] C. Nicolas Nicolaz, M. Zhadobov, F. Desmots, A. Ansart, R. Sauleau, D. Thouroude, D. Michel, Y. Le Dréan, Study of narrow band millimeter-wave potential interactions with endoplasmic reticulum stress sensor genes, Bioelectromagnetics 30 (2009) 365–373.
[18] N.J. Millenbaugh, C. Roth, R. Sypniewska, V. Chan, J.S. Eggers, J.L. Kiel, R.V. Blystone, P.A. Mason, Gene expression changes in the skin of rats induced by prolonged 35 GHz millimeter-wave exposure, Radiat. Res. 169 (2008) 288–300.
[19] C. Le Quément, C. Nicolas Nicolaz, M. Zhadobov, F. Desmots, R. Sauleau, M. Aubry, D. Michel, Y. Le Dréan, Whole-genome expression analysis in primary human keratinocyte cell cultures exposed to 60 GHz radiation, Bioelectromagnetics 33 (2012) 147–158.
[20] A. Beneduci, G. Chidichimo, R. De Rose, L. Filippelli, S.V. Straface, S. Venuta, Frequency and irradiation time-dependant antiproliferative effect of low-power millimeter waves on RPMI 7932 human melanoma cell line, Anticancer Res. 25 (2005) 1023–1028.
[21] A. Beneduci, G. Chidichimo, S. Tripepi, E. Perrotta, Transmission electron microscopy study of the effects produced by wide-band low-power millimeter waves on MCF-7 human breast cancer cells in culture, Anticancer Res. 25 (2005) 1009–1013.
[22] A. Beneduci, G. Chidichimo, S. Tripepi, E. Perrotta, F. Cufone, Antiproliferative effect of millimeter radiation on human erythromyeloid leukemia cell line K562 in culture: ultrastructural- and metabolic-induced changes, Bioelectrochemistry 70 (2007) 214–220.
[23] A. Beneduci, Evaluation of the potential in vitro antiproliferative effects of millimeter waves at some therapeutic frequencies on RPMI 7932 human skin malignant melanoma cells, Cell Biochem. Biophys. 55 (2009) 25–32.
[24] G.W. Wu, X.X. Liu, M.X. Wu, J.Y. Zhao, W.L. Chen, R.H. Lin, J.M. Lin, Experimental study of millimeter wave-induced differentiation of bone marrow mesenchymal stem cells into chondrocytes, Int. J. Mol. Med. 23 (2009) 461–467.
[25] X. Li, M. Du, X. Liu, M. Wu, H. Ye, J. Lin, W. Chen, G. Wu, Millimeter wave treatment inhibits NO-induced apoptosis of chondrocytes through the p38MAPK pathway, Int. J. Mol. Med. 25 (2010) 393–399.
[26] X. Li, M. Du, X. Liu, W. Chen, M. Wu, J. Lin, G. Wu, Millimeter wave treatment promotes chondrocyte proliferation by upregulating the expression of cyclin-dependent kinase 2 and cyclin A, Int. J. Mol. Med. 26 (2010) 77–84.
[27] X. Li, H. Ye, F. Yu, L. Cai, H. Li, J. Chen, M. Wu, W. Chen, R. Lin, Z. Li, C. Zheng, H. Xu, G. Wu, X. Liu, Millimeter wave treatment promotes chondrocyte proliferation via G1/S cell cycle transition, Int. J. Mol. Med. 29 (2012) 823–831.
[28] L. Xia, Q.L. Luo, H.D. Lin, J.L. Zhang, H. Guo, C.Q. He, The effect of different treatment time of millimeter wave on chondrocyte apoptosis, caspase-3, caspase-8, and MMP-13 expression in rabbit surgically induced model of knee osteoarthritis, Rheumatol. Int. 32 (2012) 2847–2856.
[29] X. Li, H. Ye, L. Cai, F. Yu, W. Chen, R. Lin, C. Zheng, H. Xu, J. Ye, G. Wu, X. Liu, Millimeter wave radiation induces apoptosis via affecting the ratio of Bax/Bcl-2 in SW1353 human chondrosarcoma cells, Oncol. Rep. 27 (2012) 664–672.
[30] G. Wu, T. Sferra, X. Chen, Y. Chen, M. Wu, H. Xu, J. Peng, X. Liu, Millimeter wave treatment inhibits the mitochondrion-dependent apoptosis pathway in chondrocytes, Mol. Med. Rep. 4 (2011) 1001–1006.
[31] K.K. Kesari, J. Behari, Microwave exposure affecting reproductive system in male rats, Appl. Biochem. Biotechnol. 162 (2010) 416–428.
[32] M.K. Logani, I. Szabo, V. Makar, A. Bhanushali, S. Alekseev, M.C. Ziskin, Effect of millimeter wave irradiation on tumor metastasis, Bioelectromagnetics 27 (2006) 258–264.
[33] V. Makar, M. Logani, I. Szabo, M.C. Ziskin, Effect of millimeter waves on cyclophosphamide induced suppression of T cell functions, Bioelectromagnetics 24 (2003) 356–365.
[34] V.R. Makar, M.K. Logani, A. Bhanushali, S.I. Alekseev, M.C. Ziskin, Effect of cyclophosphamide and 61.22 GHz millimeter waves on T-cell, B-cell, and macrophage functions, Bioelectromagnetics 27 (2006) 458–466.
[35] V.R. Makar, M.K. Logani, A. Bhanushali, M. Kataoka, M.C. Ziskin, Effect of millimeter waves on natural killer cell activation, Bioelectromagnetics 26 (2005) 10–19.
[36] K.V. Lushnikov, Y.V. Shumilina, V.S. Yakushina, A.B. Gapeev, V.B. Sadovnikov, N.K. Chemeris, Effects of low-intensity ultrahigh frequency electromagnetic radiation on inflammatory processes, Bull. Exp. Biol. Med. 137 (2004) 364–366.
[37] A.B. Gapeev, K.V. Lushnikov, I.V. Shumilina, N.K. Chemeris, Pharmacological analysis of anti-inflammatory effects of low-intensity extremely high-frequency electromagnetic radiation, Biofizika 51 (2006) 1055–1068.
[38] A.B. Gapeyev, E.N. Mikhailik, N.K. Chemeris, Anti-inflammatory effects of low-intensity extremely high-frequency electromagnetic radiation: frequency and power dependence, Bioelectromagnetics 29 (2008) 197–206.
[39] A.B. Gapeyev, E.N. Mikhailik, N.K. Chemeris, Features of anti-inflammatory effects of modulated extremely high-frequency electromagnetic radiation, Bioelectromagnetics 30 (2009) 454–461.
[40] R.K. Sypniewska, N.J. Millenbaugh, J.L. Kiel, R.V. Blystone, H.N. Ringham, P.A. Mason, F.A. Witzmann, Protein changes in macrophages induced by plasma from rats exposed to 35 GHz millimeter waves, Bioelectromagnetics 31 (2010) 656–663.
[41] I. Szabo, M.A. Rojavin, T.J. Rogers, M.C. Ziskin, Reactions of keratinocytes to in vitro millimeter wave exposure, Bioelectromagnetics 22 (2001) 358–364.
[42] M.K. Logani, S. Alekseev, M.K. Bhopale, W.S. Slovinsky, M.C. Ziskin, Effect of millimeter waves and cyclophosphamide on cytokine regulation, Immunopharmacol. Immunotoxicol. 34 (2012) 107–112.
[43] T.I. Usichenko, H. Edinger, V.V. Gizhko, C. Lehmann, M. Wendt, F. Feyerherd, Low-intensity electromagnetic millimeter waves for pain therapy, Evid.-Based Complement. Alternat. Med. 3 (2006) 201–207.
[44] M.A. Rojavin, A.A. Radzievsky, A. Cowan, M.C. Ziskin, Pain relief caused by millimeter waves in mice: results of cold water tail flick tests, Int. J. Radiat. Biol. 76 (2000) 575–579.
[45] M.C. Ziskin, Physiological mechanisms underlying millimetre wave therapy, in: S.N. Ayrapetyan, M.S. Markov (Eds.), Bioelectromagnetics, Springer, 2006, pp. 241–251.
[46] V.P. Lysenyuk, I.Z. Samosyuk, Y.N. Kulikovich, A.K. Kozhanova, Experimental study on the low-intensity millimeter-wave electro-magnetic stimulation of acupuncture points, Acupunct. Electro-Ther. Res. 25 (2000) 91–99.
[47] V.V. Vorobyov, R.N. Khramov, Hypothalamic effects of millimeter wave irradiation depend on location of exposed acupuncture zones in unanesthetized rabbits, Am. J. Chin. Med. 30 (2002) 29–35.

[48] T.I. Usichenko, O.I. Ivashkivsky, V.V. Gizhko, Treatment of rheumatoid arthritis with electromagnetic millimeter waves applied to acupuncture points – a randomized double blind clinical study, Acupunct. Electro-Ther. Res. 28 (2003) 11–18.
[49] A.A. Radzievsky, M.A. Rojavin, A. Cowan, S.I. Alekseev, M.C. Ziskin, Hypoalgesic effect of millimeter waves in mice: dependence on the site of exposure, Life Sci. 66 (2000) 2101–2111.
[50] A.A. Radzievsky, M.A. Rojavin, A. Cowan, S.I. Alekseev, A.A. Radzievsky Jr., M.C. Ziskin, Peripheral neural system involvement in hypoalgesic effect of electromagnetic millimeter waves, Life Sci. 68 (2001) 1143–1151.
[51] M.A. Rojavin, M.C. Ziskin, Electromagnetic millimeter waves increase the duration of anaesthesia caused by ketamine and chloral hydrate in mice, Int. J. Radiat. Biol. 72 (1997) 475–480.
[52] S.I. Alekseev, O.V. Gordiienko, A.A. Radzievsky, M.C. Ziskin, Millimeter wave effects on electrical responses of the sural nerve in vivo, Bioelectromagnetics 31 (2010) 180–190.
[53] P.H. Siegel, V. Pikov, Impact of low intensity millimetre waves on cell functions, Electron. Lett. 46 (2010) S70–S72.
[54] V. Pikov, X. Arakaki, M. Harrington, S.E. Fraser, P.H. Siegel, Modulation of neuronal activity and plasma membrane properties with low-power millimeter waves in organotypic cortical slices, J. Neural Eng. 7 (2010) 045003.
[55] I. Szabo, J. Kappelmayer, S.I. Alekseev, M.C. Ziskin, Millimeter wave induced reversible externalization of phosphatidylserine molecules in cells exposed in vitro, Bioelectromagnetics 27 (2006) 233–244.
[56] M. Zhadobov, R. Sauleau, V. Vie, M. Himdi, L. Le Coq, D. Thouroude, Interactions between 60 GHz millimeter waves and artificial biological membranes: Dependence on radiation parameters, IEEE Trans. Microwave Theory Tech. 54 (2006) 2534–2542.
[57] A. Ramundo-Orlando, G.P. Gallerano, P. Stano, A. Doria, E. Giovenale, G. Messina, M. Cappelli, M. D'Arienzo, I. Spassovsky, Permeability changes induced by 130 GHz pulsed radiation on cationic liposomes loaded with carbonic anhydrase, Bioelectromagnetics 28 (2007) 587–598.
[58] A. Ramundo-Orlando, G. Longo, M. Cappelli, M. Girasole, L. Tarricone, A. Beneduci, R. Massa, The response of giant phospholipid vesicles to millimeter waves radiation, Biochim. Biophys. Acta 1788 (2009) 1497–1507.