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Original Paper

The State of Intracellular Molecular Regulators during the Reconvalescence of Community-Acquired Pneumonia under the Influence of Microwaves at 1 GHz

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Abstract
We evaluated the levels of protein p53, retinoblastoma protein (RB), β-catenin, SMAD2, protein kinases AKT and focal adhesion kinase (FAK), and transcription factor CREB using ELISA in mononuclear leukocytes of patients with community-acquired pneumonia on days 15–17 of the disease. The research results showed that the subclinical immune-inflammatory process was characterized by a higher content of β-catenin by 19.8%, CREB by 23.3%, RB protein by 14.7%, increased phosphorylation level of protein kinase FAK by 19.8%, AKT1 at serine-473 by 65.6%, and RB by 13.7%. In the cells, there was a decrease in p53 of 15.7%, SMAD2 of 16.9%, and protein kinase AKT1 of 31.2%. Three hours after 1-GHz microwave irradiation, MNCs displayed a statistically significant increase in their content of the p53 protein of 20.0‰, RB of 9.35‰, β-catenin of 10.9‰, protein kinase FAK of 10.0‰, and CREB of 8.55‰. A day after a single irradiation of the cultures of whole blood cells, in irradiated cells compared to nonirradiated control, we observed a statistically significant increase in the content of p53 of 24.2‰, β-catenin of 15.1‰, SMAD2 of 20.4‰, RB of 9.8‰, as well as a reduction of the initially elevated levels of protein kinase AKT1 of 25.1‰, its phosphorylated form at serine-473 of 16.5‰, and a decrease in the phosphorylated form of the transcription factor CREB at serine-133 of 4.1‰. The research results indicate that a low-intensity microwave frequency of 1 GHz can be considered as a factor in molecular immune rehabilitation in conditions of community-acquired pneumonia.

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Introduction

One of the important regulatory factors controlling cellular metabolism, in particular angiogenesis, glucose metabolism, and cell cycle, is the family of proteins protein kinase B, in which the protein kinase AKT1 is the key enzyme involved in the regulation of proliferation, cell growth, and survival, cell transport and glucose metabolism [1, 2].

To control the various cellular processes, one of the most important tumor suppressors, protein p53, is also involved. It is a transcription factor against a number of specific genes providing cell cycle control. It has been shown that p53 expression is increased in response to increased expression of proto-oncogenes Ras, Myc, β-catenin, as well as the adenovirus E1A oncogene [3, 4].

Functional activity and the impact on cellular metabolism of protein p53 correspond to the functional state of the signaling pathway providing transmission of signals mediated by TGF-β, in which SMAD proteins are involved. Activation of the signaling pathway is accompanied by formation of the transcription complex consisting of SMAD-2, -3, -4, which provides the synthesis of inhibitors of cyclin-dependent kinases (CDK): CDK proteins p21, p15, and p27, causing suppression activity CDK-2, -4, -6, and related to them cyclins D and E, which results in cell cycle arrest in the G0/G1 phase and blocking the passage of the synthetic phase of the cell [5–8].

Blocking the activity of complexes CDK cyclins D and E leads to dephosphorylation of the retinoblastoma protein (RB) and the activation of suppression of its effects on cell proliferation, which is accompanied by cell cycle arrest in the G1 phase due to connection and inactivating ETS, E2F, UBF, c- Abl, and ATF/CREB transcription factors [6, 9, 10].

Thus, the transcription factor CREB, for which protein RB performs the regulatory function, provides the activation of one of the early genes, c-fos, and its immediate response and also regulates the synthesis of neuropeptides, growth factors that regulate the functional activity of nonhematopoietic cells such as neurons [6, 10].

Providing cellular system adaptation to environmental conditions, contributing to the improvement of cellular plasticity and resistance to external factors, these factors are promising targets for correction of pathological processes which are the basis of malignant transformation and autoimmune and chronic inflammatory processes. However, the states of these mechanisms in the postclinical stages of pathological processes and their role in the sanogenetic program realization have been insufficiently investigated [6, 11].

The results of biophysical studies indicate that the systemic factor of influence on the molecular processes in cells is low-intensity electromagnetic radiation (EMR). It provides the normalization of biophysical properties of the aqueous components of the internal environment [12, 13]. It has been shown that during the life of an organism, there is a change in the molecular structure of the aqueous component associated with a variety of pathological conditions [12, 14].

The impact on the water environment of low-intensity EMR frequency of 1 GHz, as one of the natural frequencies of oscillation of the water molecules, restores the broken structure of the aquatic environment during cell metabolism with the normalization of molecular, biochemical, and morphological changes [15–19].

The frequencies, uncharacteristic to fluctuations inherent to water molecules, have an adverse effect on the processes occurring in cells [12, 20]. Particularly sensitive to the action of a variety of unfavorable factors, including EMR, are organisms located in the nonstationary conditions, i.e. in conditions of transition from the state of health to the state of disease, or vice versa [11, 13].

Thus, contributing to a change in the molecular-wave balance of intracellular environment, the EMR effects on cells can be manifested by normalization, as well as by dysregulation of their functional state [12, 14, 20].

Thus, the influence of the low-intensity radiation at a frequency of 1 GHz on water media is one of the natural oscillation frequencies of water molecules. It provides restoration of the broken structure of the water media in the process of metabolic activity of cells accompanied by normalization of molecular, biochemical, and morphological changes characteristic of various pathological processes [15–19]. In this regard, the universally recognized role of microwaves used in the integrative approach to identify and refine the mechanisms of regulation of such conditions as tumor transformation, inflammation, apoptosis and autophagy, and aging should be noted [11–15]. In this case, the choice of the subject of our study, in particular the study of the features of the influence of low-intensity microwaves at a frequency of 1 GHz on cells is due to the resonance nature of the interaction of radiation with intracellular molecular targets localized in the aqueous phase. Being one of the natural oscillation frequencies of water molecules, radiation at a frequency of 1 GHz contributes to the restoration of the structure of the water media, while normalizing the molecular, biochemical, and morphological changes in cellular metabolism [11, 14]. Currently, it is not known if other frequencies could be used in medical or biological research in the range of ultrahigh or ultrahigh frequencies, which differ in the resonant nature of the effect on water media [14, 17, 18].

The purpose of this research is to study intracellular molecular regulators in the blood cells of patients with subclinical inflammation under the influence of low-intensity radiation at a frequency 1 GHz. The Ethics Committee of the Medical Institute, Tula State, approved the protocol of the present survey.

Materials and Methods

The main group consisted of 30 male patients aged 20–35 years (mean age 22.5 ± 2.2 years) with community-acquired bacterial pneumonia (CAP), a moderate course, in the stage of reconvalescence (15–17 days). The control group consisted of 15 healthy young men from among the blood donors, aged 20–33 years (mean age 22.5 ± 2.2 years).

The criteria for inclusion of patients in the study were: lack of fever for 7 days or longer; reduction of the volume infiltrative changes by not less than 2/3 from the initial values in accordance with the X-ray data; serum C-reactive protein concentration in the range of 15–20 mg/L; normalization of leukocytes in the peripheral blood; preservation of asthenic manifestations (labile blood pressure, heart rate, weakness, fatigue), and minimal respiratory manifestations (dry cough).

The material of this study was the venous blood from the cubital vein sampled in the morning (from 7:00 to 7:30 a.m.). By separating the blood sample into two parts, two subgroups in each group were formed. The first subgroup (1) included nonirradiated blood samples, and the second subgroup consisted of samples which had undergone irradiation at a power flux density 0.1 mW/cm^2 [21].

To work with cultures of whole blood cells, the sets of Cytokine-Stimulus-Best (CJSC Vector Best, Novosibirsk) were used. For this study, 1 mL of the patient's whole blood was added to the vial containing 4 mL of DMEM aquatic medium, and then the blood samples of the 1st subgroup were irradiated for 45 min in a microwave therapy apparatus Aquaton-02 (registration certificate No. FSR 2011/10939) [22, 23]. After the irradiation, the vials were placed in an incubator at 37 °C for subsequent isolation of the mononuclear cells (MNCs) using Vacutainer tubes (Becton Dickinson, USA) containing 2.0 mL of Ficoll (ρ = 1.077) and the separating gel.

The thus separated MNCs were washed twice in phosphate-buffered saline, after which they were lysed using a buffer of the following composition: 10 mM Tris, pH 7.4; 100 mM NaCl,

Table 1. The content of the studied factors in groups

Factor	Control group				Main group				Δ, %
	x	q25	Me	q75	*x*	q25	Me	q75	
β-Catenin, ng/mL	3.88	3.73	3.88	4.04	4.65	4.21	4.43	5.23	19.8
p53, ng/mL	2.94	2.84	2.94	3.04	2.48	2.21	2.47	2.59	–15.6
FAK, ng/mL	3.35	2.88	3.36	3.83	3.61	2.73	3.66	4.18	7.8
FAK (pY397), U/ng	0.49	0.55	0.49	0.45	0.59	0.61	0.63	0.61	19.8
AKT1, ng/mL	3.56	3.45	3.56	3.68	2.45	2.14	2.46	2.84	–31.2
AKT1 (pT308), U/ng	0.70	0.68	0.70	0.72	1.26	1.15	1.09	1.33	79.0
AKT1 (pS473), U/ng	0.72	0.70	0.73	0.75	1.20	1.20	1.19	1.18	65.6
CREB, ng/mL	1.20	1.17	1.21	1.24	1.48	1.17	1.43	1.76	23.3
CREB (pS133), U/ng	1.34	1.24	1.33	1.42	1.22	1.18	1.24	1.33	–9.4
SMAD2	2.61	2.56	2.62	2.66	2.17	2.08	2.18	2.27	–16.9
SMAD2 (pSpS465/467), U/ng	1.18	1.13	1.22	1.24	1.05	0.91	1.03	1.19	–11.3
RB, ng/mL	3.57	3.28	3.57	3.87	4.10	3.83	3.99	4.28	14.8
RB (pS780), U/ng	0.78	0.80	0.78	0.76	0.89	0.86	0.89	0.92	13.7

Δ indicates the difference in the studied factors between patients of the main group and the control group (%).

1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate, 1 mM PMSF (0.3 M solution matrix in DMSO). 1% protease inhibitor cocktail (Sigma-Aldrich, USA) was added (ex temporo) in a lysing solution, after which it was incubated on ice (at t = +4–5 °C) for 15 min. 1 mL of cell suspension containing 1 × 10^6 cells was used to prepare the lysates. The cell count and viability analysis were carried out using the counter TC20 (Bio-Rad, USA). Cell viability of the prepared cultures was more than 90%. The thus prepared nuclear-cytoplasmic lysates of the MNCs were centrifuged for 10 min at 15,000 rev/min with subsequent aliquoting and freezing at –76 °C.

Using the ELISA method, we determined in lysates of the MNCs the concentration of β-catenin, p53 protein, the general form of the focal adhesion kinase (FAK), as well as its forms phosphorylated in threonine at position 397 (FAK [pY397]), the total form SMAD protein and its forms (SMAD2 [pSpS465/467]), the total and phosphorylated forms of transcription factor CREB (CREB [pS133]) at serine-133 , the total and phosphorylated forms of RB at serine-780 (RB [pS780]), and total phosphorylated threonine at position 308 (AKT1 [pT308]) and serine at position 473 in the protein kinase AKT1 (AKT1 [pS473]). The concentration of studied protein kinases was estimated in ng/mL. The phosphorylation activity was evaluated in arbitrary units per ng protein (U/ng).

During the research, the reagent sets CUSABIO BIOTECH (China) were used. Analysis was performed by means of the analyzer Personal LAB (Adaltis Italia S.p.A., Italy).

Statistical processing was performed using the STATISTICA 7.0 program. Statistical significance (p) of the intergroup differences in the independent samples was evaluated using the Mann-Whitney U test, in the associated samples using a sign test. A p value <0.05 indicated statistically significant difference.

Results and Discussion

The content of the studied factors in healthy subjects and patients with the subclinical infectious-inflammatory process is presented in Table 1.

Table 2. Level of the studied factors in the MNCs 3 h after irradiation

Factor	Subgroups								Δ, ‰
	1, nonirradiated cells				2, irradiated cells				
	x	Q25	Me	Q75	*x*	Q25	Me	Q75	
β-Catenin, ng/mL	4.6	4.22	4.29	5.22	4.65	4.28	4.35	5.28	10.9*
p53, ng/mL	2.5	2.24	2.59	2.68	2.55	2.28	2.64	2.73	20.0*
FAK, ng/mL	2.99	2.77	3.15	3.3	3.02	2.79	3.17	3.33	10.0*
FAK (pY397), U/ng	0.498	0.473	0.463	0.509	0.5	0.477	0.47	0.511	4.0
AKT1, ng/mL	3.27	2.57	3.19	3.86	3.25	2.55	3.17	3.84	–6.1
AKT1 (pT308), U/ng	0.385	0.393	0.392	0.373	0.385	0.392	0.391	0.37	0.0
AKT1 (pS473), U/ng	0.385	0.393	0.386	0.409	0.385	0.392	0.382	0.406	0.0
CREB, ng/mL	2.34	2.13	2.29	2.67	2.36	2.15	2.32	2.69	8.6*
CREB (pS133), U/ng	1.218	1.192	1.258	1.165	1.216	1.195	1.25	1.164	–1.6
SMAD2, ng/mL	3.12	2.76	3.12	3.65	3.14	2.77	3.14	3.66	6.4
SMAD2 (pSpS465/467), U/ng	0.814	0.79	0.814	0.786	0.815	0.794	0.812	0.787	1.2
RB, ng/mL	3.21	2.82	3.33	3.63	3.24	2.85	3.36	3.66	9.4*
RB (pS780), U/ng	1.065	1.053	0.991	1.124	1.068	1.049	0.997	1.126	2.8

Δ indicates a difference in the index of the irradiated compared with nonirradiated cultures (‰). * $p < 0.05$, the level of significance of the revealed differences.

The analysis has shown that the resolution of the main manifestations of the inflammatory process in the reconvalescent CAP is accompanied by an increase in the intracellular concentration of β-catenin of 19.8% ($p = 0.0003$), in FAK general form of 7.8% ($p = 0.48$), its phosphoform of 19.8% ($p = 0.022$), increase in the concentration of AKT1 phosphorylated at threonine-308 of 79.0% ($p = 0.41$), phosphorylated at serine-473 of 65.6% ($p = 0.14$), and CREB factor of 23.3% ($p = 0.017$). Moreover, in the reconvalescent CAP, we observed an increase in RB protein levels of 14.7% ($p = 0.002$) and its phosphorylated form at serine-780 of 13.7% ($p = 0.00002$).

Against this background, the p53 protein level and the general form AKT1 kinase in the MNCs was reduced by 15.7% (0.0005) and 31.2% ($p = 0.00002$), respectively. We noted a decrease in the SMAD2 protein content and its phosphorylated form at serine-465/467 of 16.9% ($p = 0.006$) and 11.3% ($p = 0.52$), respectively, and the phosphorylated form of CREB at serine-133 of 9.4% ($p = 0.19$).

The obtained results indicate significant molecular changes in the MNCs characterizing subclinical immune-inflammatory process. In particular, the postclinical stage of the infectious-inflammatory process in patients with CAP is characterized by a decrease in the transcriptional activity regulated by CREB, an increase in the activity of AKT/mTOR-signaling pathway, p53 deficiency, and the tendency to reduce the reactivity of cells to TGF-β action.

The study of the early stage of the biological effects of radiation (during 3 h of incubation) is presented in Table 2.

Evaluation of the biological effects of low-intensity radiation at 1 GHz during 3 h after exposure indicates the higher sensitivity to radiation of p53 protein content in the cell. It was found that a single irradiation stimulates the process of transcription and translation of genetic information due to which an increase in intracellular concentration of the studied regulatory proteins is provided. In this case, a relatively short single exposure is able to leave a visible molecular trace, which suggests an important role of radiation in the regulation of intracellular processes.

Table 3. Level of the studied factors in the MNCs a day after irradiation

Factor	Subgroups								Δ, ‰
	1, nonirradiated cells				2, irradiated cells				
	x	Q25	Me	Q75	*x*	Q25	Me	Q75	
β-Catenin, ng/mL	4.65	4.21	4.43	5.23	4.72	4.29	4.49	5.29	15.1*
p53, ng/mL	2.48	2.21	2.47	2.59	2.54	2.27	2.54	2.65	24.2*
FAK, ng/mL	3.61	2.73	3.66	4.18	3.67	2.78	3.71	4.24	16.6*
FAK (pY397), U/ng	0.59	0.612	0.626	0.612	0.594	0.615	0.631	0.616	6.8
AKT1, ng/mL	2.45	2.14	2.46	2.84	2.39	2.09	2.4	2.8	–24.5*
AKT1 (pT308), U/ng	0.735	0.645	0.72	0.824	0.732	0.627	0.717	0.818	–4.1
AKT1 (pS473), U/ng	0.604	0.547	0.581	0.62	0.594	0.531	0.575	0.607	–16.6*
CREB, ng/mL	2.94	2.56	2.92	3.36	3.0	2.62	2.98	3.41	20.4*
CREB (pS133), U/ng	1.048	0.961	0.914	1.125	1.043	0.958	0.913	1.12	–4.8*
SMAD2, ng/mL	3.06	2.82	3.17	3.25	3.12	2.88	3.22	3.31	19.6*
SMAD2 (pSpS465/467), U/ng	0.739	0.645	0.703	0.825	0.74	0.656	0.708	0.822	1.4
RB, ng/mL	4.1	3.83	3.99	4.28	4.14	3.88	4.04	4.32	9.8*
RB (pS780), U/ng	0.885	0.862	0.887	0.918	0.889	0.869	0.889	0.919	4.5

Δ indicates a difference in the index of the irradiated compared with nonirradiated cultures (‰). * $p < 0.05$, the level of significance of the revealed differences.

The revealed effects of microwaves during 3 h after irradiation decreased in the following order: P53 > β-catenin > FAK > RB > CREB > AKT1 > SMAD2 > FAK (pY397) > Rb (pS780) > CREB (pS133) > SMAD2 (pSpS465/467) > AKT1 (pT308) = AKT1 (pS473). Thus, the level of phosphorylation of the studied factors under the influence of radiation varies less compared with their concentration in the cell. The results of the evaluation of the studied parameters 1 day after irradiation are presented in Table 3.

The analysis revealed that a single microwave exposure is accompanied by growth of the intracellular concentration of almost all studied factors, except for the family of AKT1 proteins. Thus, the effect of irradiation on the level of phosphorylated forms of the examined proteins 24 h after exposure is as follows: AKT1 > p53 > SMAD2 > FAK > AKT1 (pS473) > β-catenin > RB > FAK (pY397) > CREB (pS133) > AKT1 (pT308) > Rb (pS780) > SMAD2 (pSpS465/467).

In this case, irradiation shows a negative impact on the level of protein kinase AKT1, which manifested not only a decrease in the concentration of protein, but also its active form. In absolute terms, radiation has the greatest effect on the content of the protein kinase AKT and p53 protein in the MNCs. The increase in the phosphorylated form of FAK in MNCs indicates the activation of the modification mechanisms of cytoskeleton status and facilitating cellular response to signals transmitted via integrin receptors.

Thus, in MNCs irradiation increases the content of the factors which decrease the activity of cell proliferation, restoring in the cell the control p53-dependent processes. The molecular effects indicating the conservation in the proinflammatory potential of the MNCs may be described as adaptive, providing greater control over the cell cycle, minimizing cell consumption of energetic and plastic substrates.

Figure 1 presents the results of the graphical analysis of the dynamics of the effects of microwave irradiation culture of whole blood cells in patients with a subclinical infectious-inflammatory process.

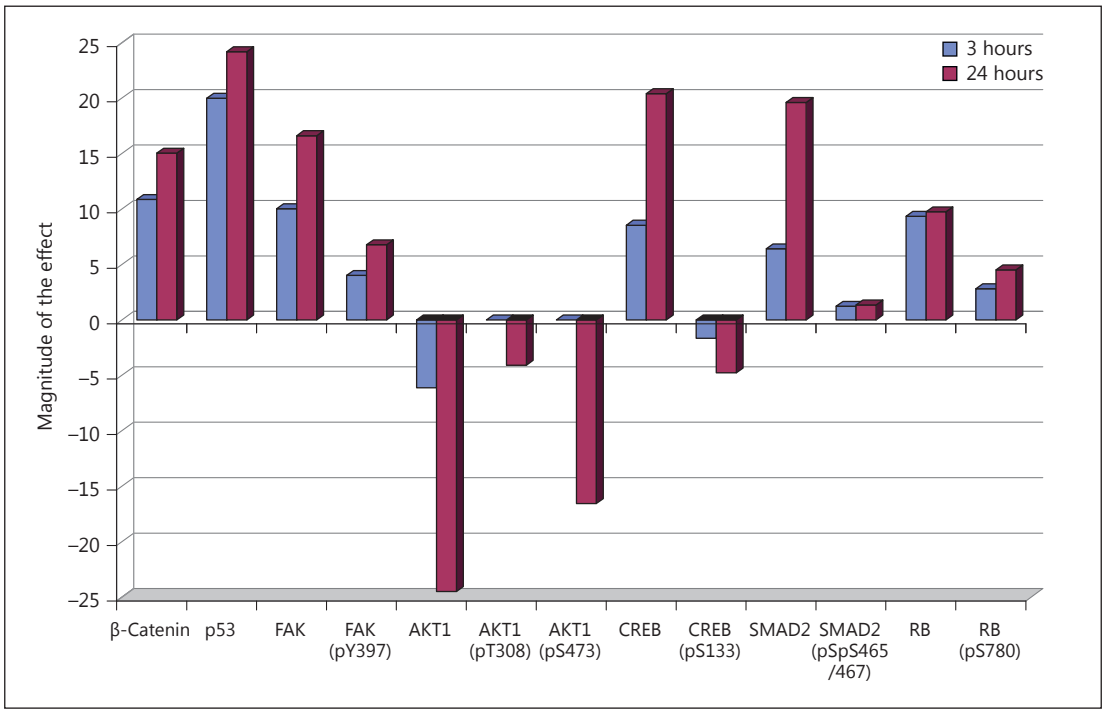

Fig. 1. The ratio of the irradiation effects 3 and 24 h after irradiation. The magnitude of the effect is the difference between average values of the content of the relevant factors in MNCs before and after irradiation (‰).

Results of the analysis demonstrated that the effect of irradiation on the content of effector molecules of the metabolic regulation in the MNCs is characterized by an increase in the biological effect. A single microwave exposure is accompanied by growth in the intracellular concentration of all studied factors except protein family AKT1 and the transcription factor CREB.

We found that the prolonged action of low-intensity microwave radiation at 1 GHz persisted for 1 day after a single exposure. The primary effect was marked 3 h after irradiation. In the studied cell cultures, a day after exposure, we noted preservation of RB protein growth stimulated by microwaves and reduction of the concentration of the transcription factor CREB. The corresponding dynamics was observed in the level of phosphorylation of these proteins. This suggests the ability of microwaves to stimulate repair and regeneration of cell damage by retarding the proliferative activity that is implemented by increasing the content of the p16 proteins, p21 proteins in cells, etc. and the factors [22].

Thus, a single exposure of the cell culture to microwaves against modulation of the proliferative activity and activation of immediate-early gene reactions (in particular, c-fos), caused by an inflammatory process, primarily, contributes to increase the content of p53 protein level in the cell. In this case, the initial irradiation effects include activation of FAK and increase in β-catenin content in the cell that increases the functional activity and immobilization of the irradiated cells [6, 22, 24, 25].

Then (to 24 h), we noted an increase in the concentration and phosphorylation of the SMAD2 factor and improvement of the signal from the TGF-β1 receptor. The cellular programs are implemented, the inflammatory activation of immune-competent cells decreases, and the reparative processes due to the polarization of macrophages towards M2 phenotype are amplified [7, 8, 22, 26].

Considering the integral role of the transcription factor CREB in the formation of cell response to the external stimuli, the dynamics of its content in the MNCs and the phosphorylation level determine the nature of the cellular response and the activity of key intracellular regulators, including mitogen-activated and stress-activated protein kinases, in the conditions of low-intensity microwave irradiation of the cells at 1 GHz [6, 22, 27, 28].

The control of cell metabolism is largely mediated by the state of the AKT/mTOR/p70S6K signaling pathway that regulates the activity of the transcription factor CREB [1, 29]. Thus, reducing the activity of protein kinase AKT1 reduces phosphorylation of CREB that suppress the transcription of immediate pre-early response gene and disables the cellular catabolic programs [1, 27, 28].

It was found that the irradiation promotes the suppression of proinflammatory activation of MNCs. Taking into account the fact that the phosphorylation level of CREB depends on the activity of protein kinases p38 JNK, ERK, MARKAR2, protein kinase A, realizing the stress-limiting strategies, the reduction of its activity in irradiated cells indicates the antistress effect of the microwaves in the relevant intracellular signaling ways [27, 28].

It has been shown that CREB plays an important role in the metabolic plasticity of the cells. Regulating the synthesis of neuropeptides (endorphin, vasopressin, VIP, etc.) and neuronal growth factor, CREB provides the plasticity of the central nervous system, the formation of synaptic connections between neurons and their regeneration, as well as the regulation of metabolism [9].

Previous studies revealed the modulating effect of microwaves on cytokine production by cells, which obviously also is realized due to changes in the level of phosphorylation of the nuclear transcription factors [23, 30, 31]. The change in the biophysical properties of histone proteins under the influence of microwaves at 1 GHz determines the possibility of epigenetic inheritance of changes initiated by the microwaves and the correction of pathological processes, in particular, the malignant transformations [32, 33].

An important result of the action of resonant radiation on the cell, being in a state of transition to normal function, is an increasing trend to the restoration of its normal reactivity that allows us to characterize the effect of the microwave at 1 GHz as sanogenetic and harmonizing [11, 17, 25, 34].

Thus, the observed changes of the intracellular content of the factors such as CREB, AKT, SMAD, and p53 allow speaking about the possibility of switching cellular strategy from the implementation of proinflammatory responses to specific regulatory responses under the influence of the resonant microwaves at 1 GHz in the course of the activity reduction of stress-activated and mitogen-activated signaling pathways [6, 7, 22, 25, 35]. Recovery of normal cell reactivity will contribute to the realization of the effects CREB, aimed at improving the plasticity of the cells to specific signals, making the cell adapt to the new conditions.

Conclusion

The subclinical course of the immune-inflammatory process in MNCs was characterized by an increase in the content of β-catenin of 19.8% ($p = 0.0003$), CREB of 23.3%, RB protein of 14.7% ($p = 0.002$), as well as by the increased phosphorylation of protein kinase FAK by 19.8% ($p = 0.022$), AKT1 phosphorylation at serine-473 by 65.6% ($p = 0.017$), and RB protein phosphorylation by 13.7% ($p = 0.00002$). However, in the cells we observed a decrease in the level of p53 protein of 15.7% ($p = 0.0005$), SMAD2 of 16.9% ($p = 0.006$), and protein kinase AKT1 of 31.2% ($p = 0.00002$). These changes are evidence of the preservation of the increased adhesion properties of MNCs at the postclinical stage of the infectious-inflammatory process, the diminution of the p53-dependent mechanisms of the cellular activity, and cellular sensitivity to TGF-β.

Three hours following exposure of MNCs to 1-GHz microwaves, we observed a statistically significant increase in the content of p53 protein of 20.0‰, RB of 9.35‰, β-catenin of 10.9‰, FAK protein of 10.0‰, and CREB of 8.55‰. One day after a single exposure of MNCs from whole blood, there was a statistically significant increase in the content of the p53 protein of 24.2‰, β-catenin of 15.1‰, SMAD2 of 20.4‰, RB of 9.8‰, as well as reduction of the initially high level of protein kinase AKT1 of 25.1‰, its phosphorylated form at serine-473 of 16.5‰, as well as the phosphorylated form of the transcription factor CREB at serine-133 compared to nonirradiated control.

The obtained results suggest that exposure of the cells to 1-GHz microwaves contributes to the normalization of their p53 protein content and the activity of protein kinase AKT1, which may be interpreted in terms of the immune-regulatory influence and be used for the purpose of molecular rehabilitation in the relevant pathological states. At the same time, the use of microwave radiation, in particular at the frequency and power level used in this study, is one of the integrative methods of applying physical factors in the area of medicine. The use of ultrahigh-frequency electromagnetic fields in biomedical technology, as well as in engineering and telecommunications technologies, is one of the most common applications of these fields that determine the relevance of ongoing research [14, 17, 25].

Disclosure Statement

The authors declare that there are no conflicts of interest regarding the publication of this article.

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