Immunotropic influence of pulse modulated 1300 MHz microwaves on cultures of lymphocytes and monocytes isolated from the blood of patients with chronic virus B hepatitis

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Abstract

The samples of mononuclear cells (PBMC) isolated from the blood of healthy donors (HD) and from the blood of patients with chronic virus B hepatitis (HV) were exposed to 1300 MHz pulse modulated microwaves at 330 pps with 5 μs pulse width, or left without irradiation. The specific absorption rate (SAR) was measured and the value of SAR = 0.18 W/kg was recorded. The microcultures of PBMC were subsequently set up to determine several parameters characterizing the T cell immunocompetence and monocyte immunogenic activity, including: proliferative response to mitogens (PHA, Con A), saturation of IL-2 receptors, T cell suppressive activity (SAT index), monocyte immunogenic activity (LM index) and production of chosen cytokines. The same absorbed dose of 1 mW/cm^2 reduced response to PHA in HD cultures and significantly increased this response in HV cultures, increased values of SAT and saturation of IL-2 receptors in the both HD and HV cultures and significantly increased production of interferon gamma (IFNγ) and production of tumor necrosis factor alpha (TNFα) in the HV cultures but not in the HD cultures. The results suggest that microwave irradiation (1300 MHz, pulse modulated) may exert distinct immunotropic influence and may enhance the effector immune response in patients with chornic virus B hepatitis, including considerable stimulation of the production IFNγ by immune cells.

Key words: *microwaves, immunoregulation, anti-viral defence*

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Introduction

The alternating changes of electric and magnetic energy of electromagnetic fields (EMF) may influence many functions of living organisms depending on the dose of absorbed energy, the frequency and the length of electromagnetic wave. Recent development of low energy and high frequency EMF emitters (mobile phones, radar and microwave broadcast stations) increased the interest on the risk of their possible harmful influence, and on the other hand, on the potential of their therapeutic application.

The undisturbed defensive, tolerogenic and proregenerative activities of immune system are commonly estimated as an important contribution of the system to homeostatic functions of the organism [1]. Thus, basic immunoregulatory activities which can be observed and precisely quantified in microcultures of immune cells separated from the human blood, represent an unique and objective model

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for investigation of possible immunotropic effects of defined EMF [2]. To determine potential immunomodulatory influences of low energy EMF the immunotropic effects of pulse modulated microwave (1300 MHz) were investigated and compared in the cultures of blood mononuclear cells from healthy donors (n=16) and from patients with chronic virus B hepatitis (n=12).

Materials and methods

The samples of mononuclear cells isolated from heparinised blood by density gradient centrifugation were suspended in RPMI 1640 supplemented with 15% autologous inactivated serum ($3x10^6$ cells/3 ml) and exposed for 1 hour to pulse modulated EMF. The microwave energy was provided by radar operating at 1300 MHz (5 μs pulse duration, 330 pps pulse repetition frequency) at average power density of 1 mW/cm^2. Before and after exposure the viability of cells was estimated and microcultures were set up in triplicates (10^5 cells/0.2 ml RPMI + 15% autologous inactivated serum). Respective triplicates of microcultures were left without stimulation or stimulated with phytohemagglutinin (PHA, HA16, 0.4 μg/culture, optimal dose, Murex Biotech Ltd, Dartford, UK) or with concanavalin A (Con A, Sigma, 8? g/culture, optimal dose) and incubated in ASSAB incubator (CO2 5%) at 37°C for 72 hours. At 24 hour of incubation the rearrangements of the cultures were performed as described elsewhere [3, 4]. For the last 18 hours of incubation the microcultures were added with 3H-thymidine (3HTdR, Amersham, UK, spec. act. 5 Ci/mM) in a dose of 0.4 μC/culture. At 72 hours the cultures were harvested and 3HTdR incorporation was measured in Packard Tri-carb 2100 TR liquid scintillation counter. The following parameters of T-lymphocytes and monocytes were measured: (a) spontaneous 3HTdR incorporation, (b) T-cell response to PHA, (c) T-cell response to Con A, (d) suppressive activity of T cells (SAT index), (e) saturation if IL-2 receptors (IL-2 index) and (f) monokine influence on T cell proliferative response (LM index). The values of indices of SAT, IL-2 receptor saturation and LM were calculated as described earlier [3, 4, 8]. Concomitantly, the samples of cell-free medium removed at 24 hour from non-stimulated cultures were frozen and subsequently assessed quantitatively by ELISA method with the use of respective Quantikine kits (R&D Systems, Abingdon, UK) for IL-1β, IL-1ra, TNFα, IFNγ and IL-10 content. The results were calculated as a mean value of dpm ± SD or as a mean value ± SD of cytokine concentration (pg/ml) for each triplicate of respective microcultures. Finally, the results were statistically analyzed using the Student t-test to estimate the significance of differences.

Results

The viability of cells tested at the end of HD or HV cultures remained at the good level of 80% of initial number of viable cells set in the culture. The exposure to EMF of HD cultures resulted in a decrease of response to PHA (67.1±8.7 x 10^3 dpm in non-exposed versus 45.8±13.7 x 10^3 dpm in exposed cultures). Reverse has been observed in HV cultures (response to PHA increased from 75.8±9.8 to 98.2±13.7 x 10^3 dpm, respectively). The response to Con A did not change in HD and HV cultures, the value of SAT index and saturation of IL-2 receptors increased significantly in the both kinds of cultures and the value of LM index increased only in the HV cultures (table 1).

The production of IL-1β and IL-1ra in non exposed cultures was lower in HD than in HV cultures. In contrast to that, the concentrations of IFNγ, TNFα and IL-10 in

Table 1. Immunomodulatory effects in PBMC cultures exposed to EMF (mean ± SD)

Test	HD cultures		HV cultures		Statistical significance
	control	EMF exposed	control	EMF exposed	
Spont. 3HTdR incorp. (dpm x 10^3)	1.9 ± 0.6	1.6 ± 0.2	2.9 ± 0.7	↓ 1.8 ± 0.3	HDc/HVc p<0.01 HVc/e p<0.01
Response to PHA (dpm x 10^3)	67.1 ± 8.7	↓ 45.8 ± 13.7	75.8 ± 9.8	↑ 98.2 ± 13.7	HDc/e p<0.01 HVc/e p<0.05
Response to Con A (dpm x 10^3)	37.2 ± 11.7	46.9 ± 2.8	40.2 ± 16.8	47.7 ± 2.4	HDc/e NS HVc/e NS
SATindex	11.7 ± 9.4	↑ 29.7 ± 7.3	19.8 ± 11.4	↑ 28.9 ± 11.8	HDc/e p<0.01 HVc/e p<0.05
Saturation of IL-2 receptors	72.3 ± 4.6	↑ 91.1 ± 11.1	72.1 ± 7.6	↑ 87.1 ± 10.4	HDc/e p<0.01 HVc/e p<0.01
LM index	5.7 ± 3.1	7.6 ± 4.2	9.7 ± 4.2	↑ 19.7 ± 8.2	HDc/e NS HVc/e p<0.01

HD: cultures of PBMC from healthy donors, HV: cultures of PBMC from patients with chronic virus B hepatitis.

culture supernatants were similar in the both HD and HV non-exposed cultures (table 2). Under influence of exposure to EMF the concentrations of IL-1β, IFNγ and TNFα increased significantly in HV cultures (respective values were: 510±212 versus 741±259, 673±92 vs. 1367±847 and 1983±936 vs. 3425±875 pg/ml). These parameters did not change in HD cultures. The concentration of IL-10 increased after exposure to EMF in HD cultures and decreased in HV cultures (table 2).

Discussion

The suspensions of lymphocytes and monocytes present in PBMC population at natural proportions, were exposed for 1 hr in pulse-modulated 1300 MHz microwaves at average power density of 1 mW/cm^2. SAR measurements performed for a physically identical HD and HV samples placed in a waveguide facility, revealed the energy absorption of 53 mW, what allowed to calculate the SAR value of 0.18 W/kg. according to the modified method of Guy et al. [5-7].

The mononuclear cells isolated from the blood represent a mixture of monocytes and various subtypes of lymphocytes, including T (CD4, CD8 and CD4,CD25 regulatory cells), B and natural killer (NK) cells. The way of cooperation of monocytes, witch belong to the APC (antigen presenting cells), and T cells, in the initiation of an immune response, greatly depends on the repertoires of produced monokines and lymphokines. The functional state of both these groups of cells can be assessed in the cultures in which response to mitogens, SAT index, saturation of IL-2 receptors and production of IL-10 and IFNγ characterize immune competence of T cells, and LM index and production of IL-1β, IL-1ra and TNFα reflect the immunogenic activity of monocytes [4, 8]. The observation of changes in these parameters, evoked by microwave irradiation of cultured cells, may therefore provide a sensitive tool estimating the potential of immunotropic influence of tested electromagnetic field.

In the both HD and HV PBMC cultures, significant, albeit not the same, functional changes were observed under the influence of exposition to 1300 MHz pulsed microwaves. In the PBMC cultures of healthy donors proliferative response of T cells to PHA decreased considerably in contrast to the improved immunoregulatory properties of T lymphocytes (increase of SAT value, saturation of IL-2 receptors and IL-10 production). Functions of T cells in HV PBMC cultures have changed in somewhat different way: response to PHA significantly increased, SAT value and IL-2 receptors saturation improved like in HD cultures, but in contrast to them, the production of IL-10 decreased.

The main differences between HD and HV PBMC cultures exposed to microwaves concerned the immunogenic function of monocytes. They did not change significantly in HD cultures, whereas in HV cultures the LM index increased from the value of 9.7±4.2 to 19.7±8.2 after the exposition, and production of pro-inflammatory monokines, IL-1β and TNFα also increased significantly. These products of monocytes are known to be active in the development and maintenance of immunogenic tissue inflammation. To exert its pro-inflammatory and immunostimulatory influence, IL-1β has to compete for access to its cellular receptor with the other monokine, the interleukin-1 receptor antagonist (IL-1ra). IL-1ra, in contrast to IL-1β, is unable to transduce the stimulatory signal. Thus, when binding to the receptor, IL-1ra prevents its activation [9-13].

Assessments of alterations in the IL-1β/IL-1ra concentration within the humoral environment of cultured cells and determination of the LM index, which value is dependent on the ratio of IL-1β/IL-1ra concentration, may provide important information on the activity of cells involved in the progression of inflammatory process [4]. In general, the higher values of the LM index and IL-1β/IL-1ra

Table 2. Cytokine production in control and EMF exposed PBMC cultures (mean ± SD)

Cytokines (pg/ml)	HD cultures		HV cultures		Statistical significance
	control	EMF exposed	control	EMF exposed	
IL-1β	287 ± 120	298 ± 189	510 ± 212	741 ± 259	HDc/e NS HVc/e p<0.05
IL-1ra	1312 ± 692	↓ 670 ± 256	2312 ± 672	2670 ± 1456	HDc/e p<0.01 HVc/e NS
IFNγ	630 ± 92	510 ± 118	673 ± 92	↑ 1367 ± 847	HDc/e NS HVc/e p<0.01
TNFα	1987 ± 986	2421 ± 475	1983 ± 936	↑ 3425 ± 875	HDc/e NS HVc/e p<0.01
IL-10	311 ± 123	↑ 623 ± 193	471 ± 149	↓ 166 ± 59	HDc/e p<0.01 HVc/e p<0.01

HD: cultures of PBMC from healthy donors, HV: cultures of PBMC from patients with chronic virus B hepatitis.

ratio characterize the activity of developmental phase, whereas decreased values of the both parameters mark the termination of immune inflammation [12-15].

The microwave stimulation of immunogenic activity of monocytes in cultures of PBMC from patients with chromic virus B hepatitis, activated in vitro the sort of a chain reaction which could play an important role in the process of in vivo elimination of viral infection. The consecutive elements of this reaction were: increased production of IL-1β and TNFα resulting in the increase of the value of LM index, subsequent enhancement of T cell proliferative response to PHA and, finally, considerable increase of the production of interferon gamma (IFNγ). Although this sequence has been observed in vitro and not confirmed in vivo, as yet, the probability can not be excluded of a similar influence of pulse modulated 1300 MHz microwaves on the immune system in vivo. Such a profile of immunotropic activity of microwaves could be beneficial in some clinical situations, for example, in stimulation of anti-tumor or anti-viral response. There is need of further investigations to determine if the immunotropic effects of 1300 MHz MW could be applied for immunotherapeutic purposes.

Conclusions

1. The features of T cell immune competence and monocyte immunogenicity in the population of mononuclear cells isolated from the human blood (PBMC) can be modulated in vitro by exposition of the cells to the influence of pulse modulated 1300 MHz microwave electromagnetic field.
2. PBMC of healthy donors and patients with chronic virus B hepatitis demonstrate in vitro different sensitivity to immunomodulatory influence of pulse modulated 1300 MHz microwaves.
3. The exposure to microwaves increased immunoregulatory poroperties of T lymphocytes in mononuclear cell cultures of healthy donors as has been demonstrated by decreased proliferative response to PHA and increased T cell suppressive activity and production of IL-10.
4. Exposition of PBMC of patients with chronic virus B hepatitis to 1300 MHz microwaves resulted in significant stimulation of immunogenic activity of monocytes (increased value of LM index, increased production of IL-1β and TNFα), enhancement of T cells response to PHA and increased production of IFNγ.
5. Electromagnetic stimulation of the production of IFNγ by immune cells of patients with chronic virus B hepatitis may prove to be useful for therapeutic purposes. The suggestion needs further investigation.

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