

Ecologically safe experimental purpose application of microwaves in the treatment of chronic neurological disorders: optimization and risks

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DOI: 10.29317/ejpfm.2018020207

Received: 03.05.2018

Current paper describes the experimental dual-purpose application of microwaves in a controlled clinical trial on optimizing therapy for chronic neurological disorders. As a part of integrated treatment, low-intensity microwave-assisted blood modification has been used. It represents a physiotherapeutic method based on the autologous blood transfusion (self-blood treatment) after the short-term exposure to microwaves ($\lambda = 7.2$ mm). Because of beneficial clinical impact of the microwave autohemotherapy previously observed, we aimed to find a possible way to track a manifestation of nonthermal microwave effects. For this purpose, we have applied a microwave single-frequency ($\lambda = 7.6$ mm) dielectricometry technique as a tool for blood sensing. Also, we used the osmotic fragility test to detect the membrane-receptor activity level using patients' red blood cells before and after the course of therapy. Our preliminary results have shown an enhancement of blood cell reactivity and a clearly defined tendency to normalizing parameters under study in those patients who received the integrated treatment. By analyzing the data obtained by different methods, we have revealed that characteristics are well correlated.

Keywords: microwave blood sensing; microwave autohemotherapy; diagnostics; permittivity; red blood cells.

Introduction

Throughout the world, the most important factor determining human health is the cleanliness of the environment, the absence of stress, the quality of population nutrition and its safety. Nowadays, electromagnetic pollution of the environment is one of the most urgent problems of mankind. It is established that people who are often exposed to stress are more often sick. In this regard, the development and implementation of preventive measures and the treatment are among the priority tasks of environmental and biological science using new methods, in particular, using microwave ovens [1,2]. At the end of the last century, the concept of microwave nonthermal effects has aroused extensive attention. Despite

numerous reports devoted to this point [1, 2], the mechanisms underlying these effects upon biological objects are still unclear.

The individual authors' research [3,4] contains theoretical bases for preparing samples in microwave ovens, and the effects of microwaves on various biological systems. A number of scientists found that microwave therapy has the following effects: anti-inflammatory, metabolic (acceleration of metabolic processes), trophic (improving blood flow) and secretory (the speed synthesis of biologically active substances). Furthermore, there are different opinions on the concept of "non-thermal effect" [3, 4]. In this regard, we are interested in the experiment designed to elucidate whether the effect of low-intensity microwave-assisted blood modification can be corroborated with other techniques, for instance with a microwave dielectric blood sensing.

Thus, we found isolated information about the results of research in terms of environmental safety in accessible domestic and foreign literature, very little information on the experimental application of microwave ovens in clinical studies to optimize therapy for chronic neurological disorders.

In our previous study [5], we have described the approach to a characterization of red blood cells (RBCs) in a case-control study by means of the microwave waveguide-based dielectrometry method. We have presented the correlation data between a drug-induced dielectric response of RBCs and their beta-adrenergic receptor (beta-AR) activity, which depends on the health status [6]. Being the major component of the interaction between the sympathetic nervous and the cardiovascular systems, beta-AR function plays a crucial role in cerebrovascular incidents too. Because activated sympathetic tone is associated with poorer outcome in certain neurological cases (for instance, after stroke), we have investigated whether the beta-AR activity is associated with lesser disease severity and with improved outcome after treatment. All this led to the need for research, and defined the purpose and objectives.

The goal of our present work is two-fold: (1) evaluating low-intensity microwave effect on the blood of patients with a mild neurological disorder, and (2) demonstrating an opportunity of microwave blood sensing for this purpose. The study is a controlled trial involving two cases: standard medical therapy and supplemented by microwave-assisted self-blood treatment (autohemotherapy). Note that in this particular case, microwaves serve as both an influencing factor and a diagnostic tool that is an interesting phenomenon in itself.

Microwaving blood. instrumentations and approaches

Dielectric Waveguide Measurements

This method is based on the measurements of complex reflection coefficient from short-circuit multilayer structure containing the sample in the single-frequency mode ($f=39.5$ GHz). Measurement setup (see Figure 1) consists of the reflectometer based on a six-port measuring line and a waveguide sensor. The latter is the most important elements of the measurement system. It is a short-circuited segment of waveguide with rectangular cross-section of 5.2×2.6 mm which holds up to 5-7 μ l of sample (sample thickness is about 100 μ m). Such configuration enables to make the reproducible microliter measurements to a precision at least of $\pm 2\%$ both in ϵ' and ϵ'' . Signal power in a waveguide transmission line does not exceed of 10 mW; measurement time is 4 seconds.

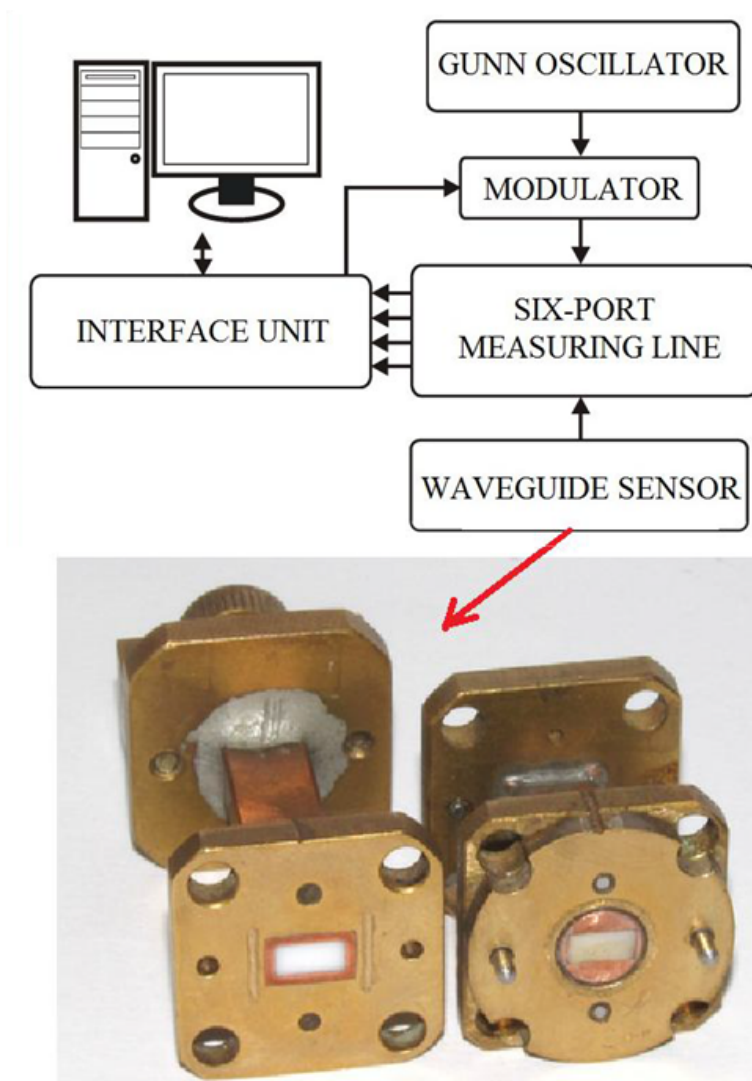


Figure 1. Measurement setup.

The outstanding feature of the sensor we use is an enhancement measuring sensitivity achieved by additional matching elements [7] that have been realized specifically for our purposes, namely, the repeated measures of high-loss biological objects. A more detailed about the measurement setup and the sensor optimization can be found in [8, 9].

Microwave Autohemotherapy Method

The autohemotherapy is a physiotherapeutic method based on the autologous blood transfusion (i.e. the collection of blood from a single patient and retransfusion back to the same patient) after exposure to some kind of external factors such as ultraviolet light [10], laser, ozone [11], etc. In this particular case, blood is exposed to low-intensity microwave irradiation.

There are a great variety of ways to apply the action of electromagnetic irradiation upon biological objects. For a microwave-assisted blood modification, the antenna-type radiators are usually employed: horn, dielectric antennas, bilifur helices, open end-faces of metal and dielectric waveguides, etc. But often these modalities tend to suffer some disadvantages. Among other things, it may not be possible to provide for homogeneity of the density of incident energy beam and the strict radiation dose; to control the external background while irradiating, and

to keep the sample in sterile environment.

The advantage of the method we used [12] is that it allows solving the above mentioned problems. The device for microwave blood activation (see Figure 2) consists of a cylindrical cavity-resonator 1 with a slot 2. The slot is connected with the rectangular waveguide of a standard cross-section 3. A single-use syringe 4 with blood is placed into cavity through the axial hole 5 and fixed in the axial groove 6. The device also contains an additional chamber 7 connected with the resonator by the beyond-cutoff hole wherein the needle with a protection cap is arranged. Groove 8 is filled with an absorber to preclude an outgoing radiation. The device operates at the radiation wavelength of $\lambda = 7.2$ mm. According to the series of researches [13] this wavelength is one of the biologically active modes, thus we have chosen it for our study. The resonator diameter is ($d = 35$ mm, the syringe diameter is $D = 22.5$ mm. The height of the cylindrical resonator is $h = 78$ mm. The excitation slot is (0.1×7.2) mm with a taper of (7.2×3.4) mm. Signal power is not more than 10 mW.

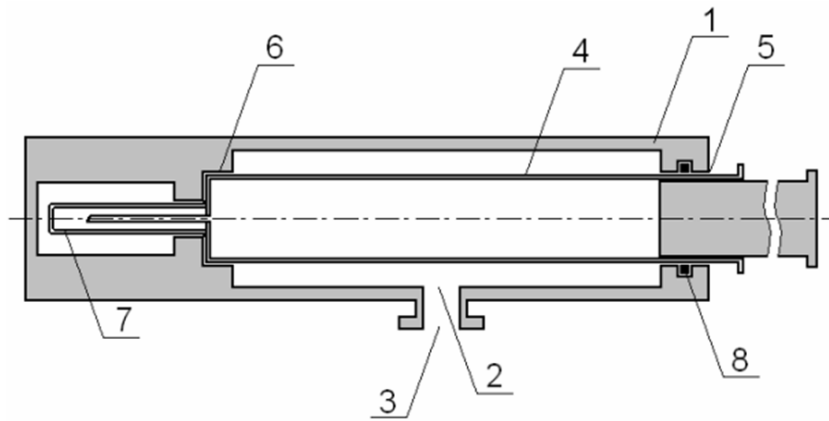


Figure 2. The sketch of the device for microwave blood irradiation (axial view) based on the cylindrical cavity resonator with a single-use syringe placed in it [12].

Figure 3 illustrates the results of electrodynamic simulation of EM field distribution while varying an inner cavity diameter D [14]. The case c) when the excitation of higher resonator mode occurs is turned out to be the most appropriate as it provides the maximum homogeneity of irradiation and the effective field interaction with an absorbing medium (blood).

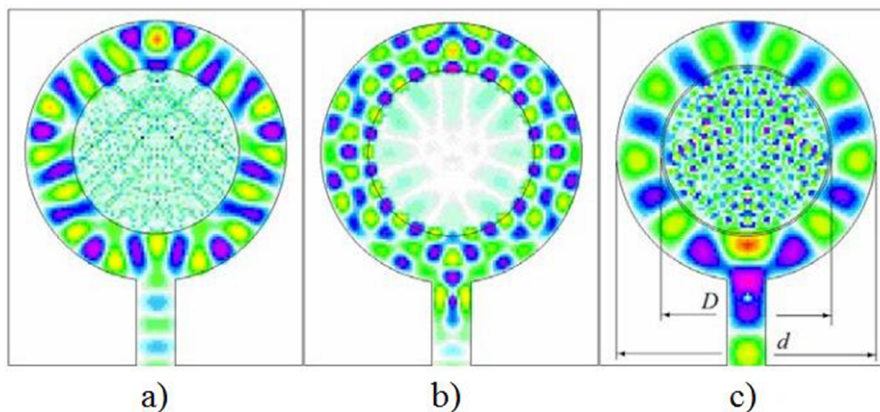


Figure 3. The results of electrodynamic simulation of the field distribution in the cross-section of a cavity resonator [14].

Research protocol

We studied blood samples of 31 patients with a mild form of hypertensive encephalopathy, which were subdivided into 2 groups (A and B) depending on the evidence or no contraindications to the autohemotherapy. Group A (n=15, aged 56.1 ± 7.3 years) consists of the patients treated with standard medical therapy; group B (n=16, aged 52.6 ± 8.6 years) consists of those who received standard treatment plus the course of autohemotherapy; comparison group (control) consists of healthy individuals (n=9, aged 55.2 ± 9.4 years) whose indicators are reference (baseline).

Because of a multidisciplinary approach to the experiment, we used different strategies of microwaving blood and samples preparation.

Microwave Autohemotherapy Procedure

The essence of this technique is as follows. A single-use syringe with patient's heparinized blood (15 ml) is placed into a resonant chamber and is exposed to microwave irradiation for 1 minute. Then an activated autoblood is transfused back to the patient. The course of the treatment includes 10 procedures under the medical care

The mechanism of low-intensity microwave effect on blood is unclear at the moment. However, there is a hypothesis explaining the beneficial clinical effects due to decreasing "effective thickness" of the membrane diffusion water layer, which, in turn, leads to the increase in permeability of cellular membranes and enzymes activity in cells [15].

Beta-Blocker-Based Osmotic Fragility Test

Osmotic fragility refers to the degree of haemolysis (rupture) that occurs when RBCs are subjected to osmotic stress by being placed in a hypotonic solution. The basis for the modified method [6] is a haemolysis inhibition in the presence of beta-blocker propranolol, which binds to beta-ARs of blood cells and reduces the degree of haemolysis. This test is commonly used to aid with diagnosis of diseases associated with RBC membrane abnormalities.

The degree of haemolysis is determined using photometry by tracing the optical density of supernatant fluid after blood centrifugation. Beta-receptors activity is numerically expressed by beta-ARM index (in arbitrary units, a.u.). It reflects the increase in osmotic resistance of cell membranes due to a beta-blocker binding. According to the guideline [6], about 93% of healthy individuals have reference values of the beta-ARM index in the range from 2 to 20. The index values and receptors activity have reciprocal correspondence: beta-ARM index will be numerically increased as the osmotic resistance is decreased, and vice versa.

Blood Samples Preparation

For dielectric and photometric measurements we used RBCs extracted from venous blood by centrifugation according to the developed lab protocol. Clotting was prevented by the addition of heparin solution (0.1 ml of 5000 USP units/ml) for a dielectric study, and EDTA (0.1 ml of 0.5 M EDTA per 5 ml of whole blood) for assessing beta-ARM index.

Microwave Dielectrometry of Red Blood Cells

The main idea of dielectric observation is to detect the change in RBCs permittivity ($\Delta\epsilon'$) induced by *in vitro* action of certain biological agents (in particular, beta-blockers). Red cell dielectric response depends on the health status of blood

donors [9], correlates well with beta-ARM index [5], and may serve as the assessment criterion of beta-ARs function [16].

To characterize beta-ARs activity the RBCs were incubated *in vitro* with beta-blocker propranolol (7.5 g/l) for 30 min at T=(23-25) °C. Permittivity measurements have been performed after the incubation immediately. All manipulations with blood were conducted during 3 hours since its sampling. Data handling and statistical analysis were performed using free software programming language R [17].

Experimental results

At the first step of the examination we compared membrane activity in all participants enrolled to the study (including controls). The pre-therapy values of beta-ARM index were distributed within the normal range for the control group - 16.1 ± 4.3 a.u., and were shared equally between the study groups - (47.7 ± 9.2) and (46.2 ± 6.8) a.u. - for A (standard treatment) and B (complex therapy), respectively. The latter data indicate low level activity of beta-ARs in patients with a chronic neurological disorder. This fact means that the target groups are well matched.

The next step was to characterize the receptor activity after the course of microwave autohemotherapy. Thus, we have repeated our study on the tenth day of the treatment, and have compared the parameters with previous ones obtained on the day of admission (see Figure 4).

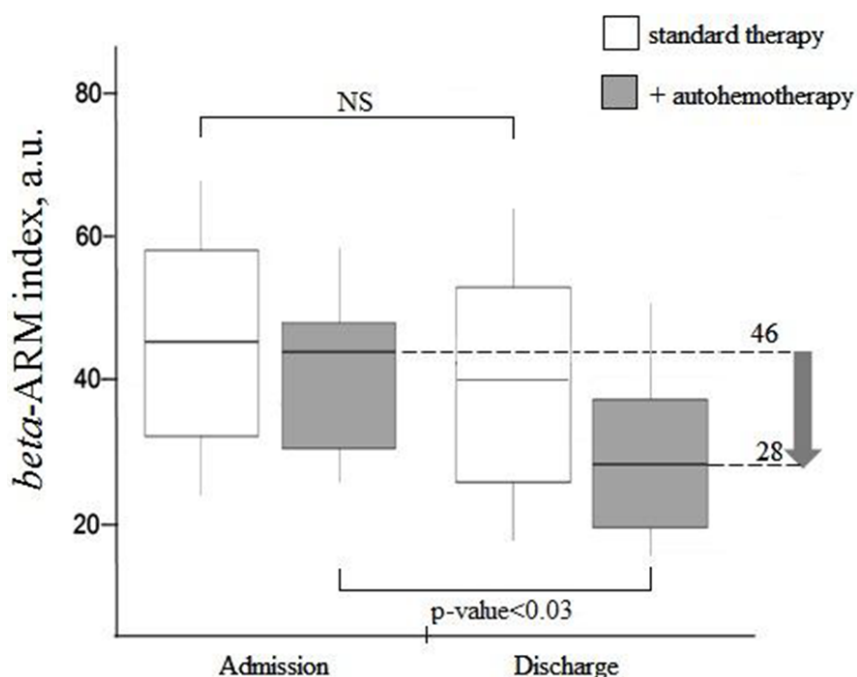


Figure 4. Experimental distribution of beta-ARM indices before and after the course of therapy between the groups A (standard treatment) and B (complex therapy). NS denotes statistically nonsignificant difference.

Inferentially, the behavior of beta-ARM indices shown in Figure 4 indicates a statistically significant ($p < 0.03$) normalizing receptors activity in the group of those patients who received complex therapy (medicated plus autohemotherapy). This finding illustrates successful therapeutic outcome and meets the result of microwave dielectric testing (Figure 5).

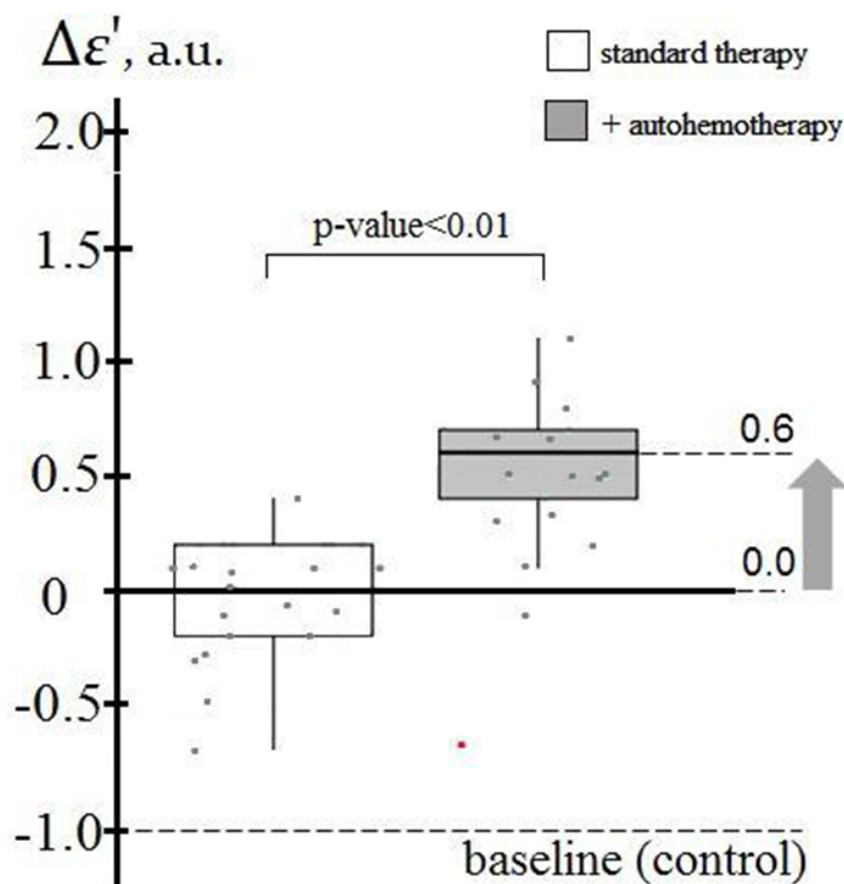


Figure 5. RBCs dielectric response ($\Delta\epsilon'$) to a beta-blocker on the 10th day of therapy. Baseline means $\Delta\epsilon'$ in health individuals as a reference group without any medical intervention.

Figure 5 illustrates the permittivity differences induced by the beta-blocker in group B compared to group A after the treatment. As Figure 5 shows the results of the dielectric study have revealed the enhancement of RBCs dielectric response to beta-blocker caused by microwave autohemotherapy. There was a highly significant ($p < 0.01$) difference in $\Delta\epsilon'$ between groups under study. However, it doesn't guarantee that the effect size observed in group B is clinically meaningful since there is still a great discrepancy between the values and sign of dielectric response in the control group (baseline) and the group B (complex treatment). Probably, dielectric observations should be repeated later, for instance on 20th or 30th day of follow-up of the patients.

Table 1.

Data changes in element composition.

Group	beta-ARM index, a.u.		$\Delta\epsilon'$, a.u.		p-value
	Before	After	Before	After	
Control	16.1*	15.3*	-1.0	-	NS*
A	47.7*	39.2*	0.06*	0.10*	NS
B	46.2*	28.1*	0.05**	0.6**	0.03*0.01**

According to the medical data obtained, microwave autohemotherapy provides beneficial action on the subjective (overall health, enhancement of mood, increment in activity, etc.) and the objective (cognitive function criteria based

on standard neurological tests) symptoms compared to medicated mono-therapy [18]. It also affects on rheological blood properties causing the advancing in RBC elasticity, cellular architectonics, and reducing red cell rigidity and aggregation rate [19].

Conclusions

The advances in microwave technologies have contributed to their widespread usage from industry and engineering to healthcare. Being contactless, label-free, and inexpensive they are particularly attractive for biomedical applications. Specific microwave properties provide a wide scope of their use both in diagnostics and therapeutics.

In this work, using multi-disciplinary approach, we combined different diagnostic techniques to evaluate therapeutic effect caused by microwave autohemotherapy in the group of patients with a mild neurological disorder.

Our preliminary results have shown the enhancement of red cells' reactivity and revealed more defined tendency to normalizing parameters in the group of patients who received integrated therapy. The data obtained by different methods are well correlated, but the clinical significance of dielectric results is now open to question. At the moment of examination, we have confirmed the prospects of the methodology we are developing, and we assume that dielectric observations in the longer term may then illuminate this issue.

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