LED SYSTEM OPTIMIZATION FOR PHOTOBIOMODULATION OF BIOLOGICAL TISSUES

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ABSTRACT

Light as electromagnetic radiation is used for the treatment and prevention of many diseases including neurodegenerative brain disorders. In recent years, non-coherent light sources such as light-emitting diodes (LEDs) and broad-band spectrum lamps have become commonly used due to the same effects as low-level laser therapy. Here, we present the design and development of a specialized system for irradiation of biological tissues that consists of a wet organ bath (WOB) installation for irradiation of the samples and three LED sources (blue, green and yellow) with intensity, power density and direction, which can be experimentally optimized. We prove that this system allows modulation of the contraction of smooth muscle cells (SMCs) under protective conditions and to potentiate their biological functions by activation of neuronal and non-neuronal serotonin controlled pathways.

Keywords: light therapy, smooth muscle cells, serotonin, 5-hydroxytryptamine (5-HT), LED.

INTRODUCTION

In medical practice, different types of light sources are applicable - from gamma irradiation in cancer treatment, X-rays in diagnostics, visible light sources in biomodulation therapy to infrared light in rehabilitation and pain relief. Light as an electromagnetic radiation is employed for the treatment and prevention of numerous diseases for years [1, 2]. Recently, it has been proven that photobiomodulation therapy benefits even treatment of neurodegenerative brain disorders like Parkinson's and Alzheimer`s diseases [3]. However, in recent years, non-coherent light sources such as LEDs and broad-band lamps have become more common. It has been established that the use of incoherent semiconductor diodes can achieve the same results as low-level laser therapy (LLLT) [4]. The advantages of LEDs include no laser safety considerations, ease of home use, ability to irradiate large areas of tissues at once, the possibility of wearable devices, and much lower cost per mW [5]. Studies show that LED mechanism of action primarily involves absorption of the light through the mitochondria, leading to increased membrane potential, electron transport, oxygen consumption, and adenosine triphosphate synthesis [6]. Moreover, different LEDs have been proven effective on cell viability, growth, and attachment characteristics of aortic endothelial cells and smooth muscle cells (SMCs) in vitro [7].

The SMCs presented in the walls of all hollow organs, including the stomach are widely used as a model system due to many receptors presented on their surfaces. This tissue culture allows high reproducibility, sensitivity and selectivity in the investigation of many biologically active components, hormones and mediators [8]. Serotonin, also called 5-hydroxytryptamine (5-HT) is one of the seven major neurotransmitters that act as a hormone and mediator in modulating mood, cognition, reward, learning, memory, and numerous physiological processes such as vomiting and vasoconstriction [9]. It is secreted by the enterochromaffin cells in the intestinal mucosa and is the main regulator of the gastrointestinal tract's functions including peristalsis, secretion, vasodilation and perception of pain or nausea [10 - 12]. These regulatory functions are mediated through the activation of a diverse family of 5-HT receptors (5-HTRs) on intrinsic and extrinsic afferent nerve fibres that are located in the lamina propria. 5-HTRs are presented in all types of tissues in the human body (mainly in the brain, bowels and thrombocytes) [13]. In the rat stomach smooth muscle (SM) tissues 5-HTRs are predominantly highly expressed [14]. Through the receptors 5-HT mediates the intercellular communication and transmission of the nerve impulses, therefore it must be kept in optimal quantities as neither low nor high levels are recommended [11]. High 5-HT levels are associated with the toxic serotonin syndrome [15]. Alternatively, its deficiency is linked with psycho- and physiological disorders [16].

Here, we present the design and development of a specialized system for irradiation of biological tissues that combines a wet chamber for tissues irradiation and three LED sources (blue, green and yellow), all of them with adjustable power density, intensity and direction. We prove that this system allows in a sophisticated way to modulate the contraction SMCs and thus to potentiate their biological functions by activation of neuronal and non-neuronal serotonin-controlled pathways.

EXPERIMENTAL

Drugs and solutions

5-HT and acetylcholine (ACh) were purchased from Sigma. The Krebs' solution (KS) was freshly prepared (Na^+ - 143 mmol L^{-1}; K^+ - 5.84 mmol L^{-1}; Ca^{2+} - 2.5 mmol L^{-1}; Mg^{2+} - 1.19 mmol L^{-1}; Cl^- - 133 mmol L^{-1}; HCO_3^- - 16.7 mmol L^{-1}; $H_2PO_4^-$ - 1.2 mmol L^{-1} and $C_6H_{12}O_6$ - 11.5 mmol L^{-1}).

Animals and breeding conditions

Male Wistar rats weighing 175 - 230 g were bred under standard laboratory conditions (temperature 22°C ± 1°C, humidity 45 %, 12 h dark/light cycle, food and water ad libitum). All animal experiments were executed under the requirements by the International Council for Ethical Guidelines for Animal Breeding Labs for Researchers, ARRIVE, and the EU Directive 2010/62/EU for animal experiments. Approvals from the Bulgarian Food Safety Agency (permission number: 229/09.04.2019) and the Ethics Committee of the Medical University - Plovdiv, Bulgaria (protocol number: 145/09.04.2019) were issued too.

Isolation of SM strips and in vitro study of SM contractility

Twenty euthanized male Wistar rats were used. Rat stomach SM strips (20.0 mm ± 1.5 mm) without mucosa were separated randomly in organ baths, prefilled with 15 mL modified KS, oxygenated with 95 % O_2 and 5 % CO_2 at standard temperature 35.5°C ± 0.3°C. The mechanical activity was amplified with a three-channel interface system for registration and investigation of spontaneous muscle contractility of the muscle strips. The normal contractile activity was recorded after the equilibration period. The baseline tone and relative change in muscle contraction were analyzed for a 5 min period. The values were defined as a predrug, baseline period and were used for further comparative analysis. ACh (10 μM) was added to the organ baths and the changes in spontaneous activity were recorded for 5 min. At the end of each trial, the organ baths were flushed with KS and 10 μM ACh was added to test the ability of the samples to exert a contractile response after activation of cholinergic receptors. To investigate the effect of 5-HT on the SM spontaneous contractile activity (SCA) we used a concentration of 0.005 mM, which corresponds to EC_{100}. 5-HT at this concentration was added in every organ bath. The above-mentioned parameters were analyzed at the same time and were represented in percentage of 10^{-5} M ACh reaction.

Optimization of the conditions for in vitro irradiation in WOB

It is compulsory the process of irradiation to be done not in a liquid but a wet media due to the specific absorption of electromagnetic irradiation in a solution with tissue samples [17]. The investigation has been performed under conditions without buffer for a period of 1 to 2 min, which has shown that no alterations in the parameters of SCA were occurred. The reactivity of the samples to ACh (10 μM) and 5-HT (5 μM), assessed 5, 10, 20, and 30 min after the exposure of the tissue to 2 min in the wob, remained unchanged ($n = 10$, $p > 0.05$).

Radiation dosing for electromagnetic irradiation, light sources - 3W LED - blue, yellow, green

The experiments were conducted with the use of LED having the constant power of 3 W and releasing different light wavelengths. The SM samples were exposed to LED sources emitting blue light corresponding to the wavelengths of 470 nm (blue), 532 nm (green) and 590 nm (yellow) for 60 sec.

System for power supply and power density meter

The system for autonomic direct current (DC) power supply was provided to each LED, allowing the proper function of the device with an adjustable current. The intensity meter measured the energy density per sec. The power density was constant for all LEDs (emitting blue, yellow and green) and its value equalled to 4 mW cm^{-2}.

Optical system and regulation of the exposure to the electromagnetic radiation

The collimation of electromagnetic energy flow was achieved through an optical system that ensured the desired power density of the LEDs. A programmable unit enabled the regulation of the exposure of the laser beam to comply with the experimental protocol.

Data quantitation and statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 17.0. The normal distribution was evaluated with One-sample Kolmogorov-Smirnov test. In the case of a normal distribution, one-way ANOVA and the Bonferroni post-hoc test were employed for multiple comparison analysis. The results were reported as mean ± SEM. The number of tested preparations is given as N. Results were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

A novel system for irradiation of biological tissues have been developed and is schematically represented in Fig. 1. The system consists of five modules: a 3W LED (blue, green, yellow), an autonomous power supply, current control, a mechanic-optical system for formation of the energy flow and an exposure regulation unit. The system was designed to allow precise positioning of the light source with the irradiated structure, allowing accurate dosing of the absorbed electromagnetic energy and optimization of the exposure time.

In the current study, we have used male Wistar rats as model organisms. SM strips have been dissected from the stomach of euthanized rats. The SM strips have been divided into four groups: the 1st contained untreated SM strips and was considered as a control group, the 2nd was composed of SM strips, irradiated with blue light, the 3rd - of SM strips, irradiated with yellow light and the 4th comprised SM strips, irradiated with a green light. The effective concentration for maximal effect (EC_{100}) for 5-HT was 5×10^{-6} M. 5-HT was an important player in our experiments. The reason for this is that 5-HT modulates human behaviour, circadian rhythms, seasonal depressions, appetite, memory, learning, the function of the cardiovascular and endocrine systems, etc. [18]. Any decrease in the 5-HT levels could be due to a low secretion, deactivation, oxidation and/or reuptake [19]. In the medical practice the increase in 5-HT levels through

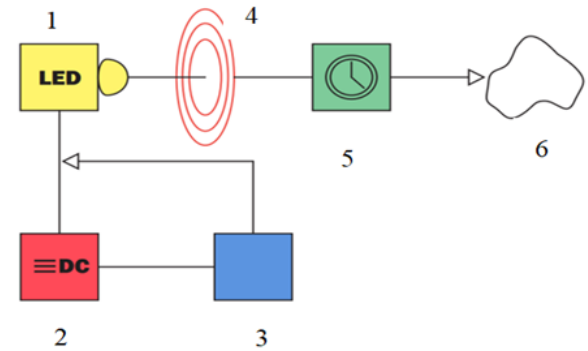

Fig. 1. System for radiation dosing for electromagnetic irradiation (1 - 3W LED (blue, green, yellow); 2 - autonomous power supply (DC); 3 - current control; 4 - mechano-optical system for formation of the energy flow; 5 - exposure regulation unit; 6 - place for the objects exposed to electromagnetic irradiation).

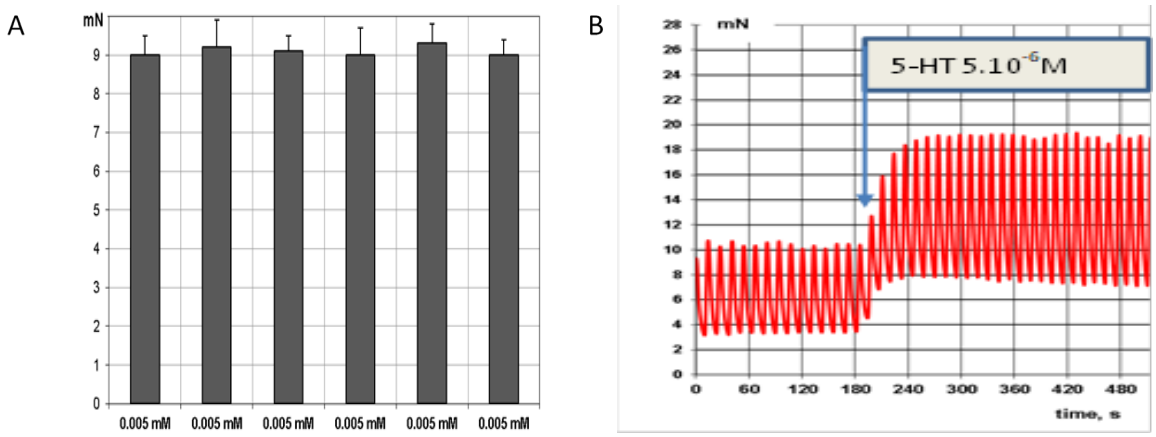

Fig. 2. Effect of 5-HT at EC_{100} on the contractile activity of rat stomach SM strips, $p > 0.01$; $n = 7$. (A - Effect of multiple applications of 5-HT at EC_{100}; B - Contractile activity of the smooth muscle strips before and after application of 5-HT).

medications as selective serotonin reuptake inhibitors (SSRI) caries potential side effects for 5-HT toxicity [9]. The commonly recommended remedies for boosting of 5-HT levels for treatment of seasonal depressions are light therapy or photobiomodulation [20]. It is known that 5-HT plays a crucial role in gastrointestinal motility too, which is confirmed by the fact that the application of 5-HT results in changes in SCA of SM tissues [21].

The contractile activity of the rat SM strips has been tested after application of 0.005 mM 5-HT, which corresponds to EC_{100}. It was analyzed and represented in percentage of 10^{-5} M ACh reaction. Fig. 2A demonstrates the results for the contractile activity of the studied rat SM strips after one to six times the application of 5-HT at a concentration of EC_{100}. The results show that 5-HT at EC_{100} does not change the contractile activity of the SM strips regardless of the frequency of its application (Fig. 2A). Fig. 2B shows the contractile activity of the smooth muscle strips before and after the treatment with 5-HT at EC_{100} (5×10^{-6} M). The abscissa represents the time measured in seconds while the ordinate - the force of contraction measured in mN.

It is well known that water absorbs electromagnetic irradiation. Our modification of classical organ bath includes a specifically designed WOB. In these conditions the SM kept their contractile activity. It was used to allow accurate irradiation of the SM strips with the three LED lights. The reactivity of the SM samples to 5-HT occurred after the exposure of the tissue to the WOB for 2 min. Significant changes in the reactivity ($n = 9$, $p > 0.05$) of the preparation to 5×10^{-6} M 5-HT at the modified condition (WOB) were not observed (Fig. 3). These results proved that the WOB system for irradiation of tissues allows accurate and temperate conditions for irradiation without any changes in the physiology of the studied tissues.

The next step was to apply the WOB for irradiation of the studied four groups of rat SM samples and to study the influence of LEDs on the 5-HT effect, applied at EC_{100}. This was provoked by the requirement the process of irradiation to be done not in a liquid but a wet media due to the specific absorption of electromagnetic irradiation in a solution with tissue samples. The SM samples were exposed to LED emitting blue light corresponding to the wavelengths of 470 nm (blue), 532 nm (green) and 590 nm (yellow) for 60 sec. Fig. 4 shows the influence of the tested LEDs on the contractile activity of the SM

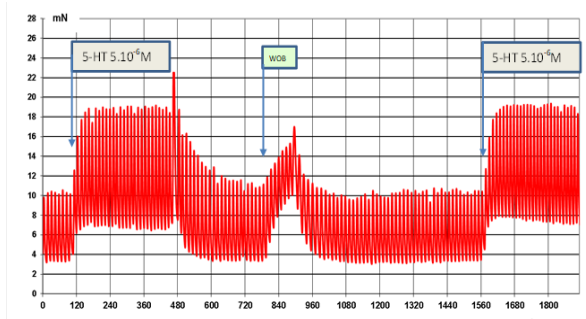

Fig. 3. Changes of the effect to 5-HT at EC_{100} concentration on the SM strips into the WOB for 1 min and a new application of 5-HT after 11 min - a representative mechanogram.

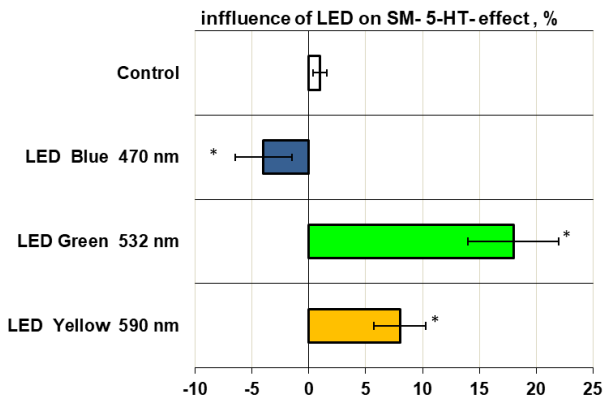

Fig. 4. An effect of 5-HT at concentration EC_{100} on the contractile activity of SM tissues estimated 20 min after their exposure for 60 sec to blue, yellow and green light with 4 mW cm^{-2} power density, $p < 0.05$; $n = 7$.

samples in response to 5-HT at a concentration of EC_{100}. The influence of the electromagnetic irradiation on the tissue samples led to a change in their reactivity to 5-HT. Comparison of each group with the other three revealed statistically significant differences ($p < 0.05$; $n = 7$).

At in vitro conditions, the neuronal reactivity of SM tissues is poorly understood. We assume that the effect of serotonin reuptake is negligibly small thus further hypothesizing that the varying effects of 5-HT after irradiation in our experimental procedures are mediated by two reasons: interaction of electromagnetic irradiation with 5-HTRs or possible influence of the electromagnetic irradiation on the enzymatic activity. The first reason is less likely to occur due to the short-term interaction of the electromagnetic radiation with the biological tissues and the relatively short time interval of 20 min for any type of cellular response, namely, a receptor expression. Therefore, we assume that the explanation lies on the second reason and this is the influence of certain enzymes that regulate the 5-HT levels, namely, the enzyme monoamine oxidase A (MAO-A) that preferably degrades 5-HT [22, 23]. This hypothesis though needs further experiments for verification.

CONCLUSIONS

We have designed and developed a specialized system for irradiation of biological tissues that allows fine modulation of the contractile abilities of SMCs and potentiates their biological functions by activation of neuronal and non-neuronal serotonin controlled pathways.

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