Innovative Light Therapy: 2. Determination of the Biological Contribution of Fullerene, as a Converter of Polarized Light, on a Model of Formalin-Induced Pain

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Abstract: Introduction: Our previous studies on the formalin pain model have shown that halogen polarized light converted by fullerene produces a significant antinociceptive effect. It was installed for applications on the locus of pain or acupuncture points (AP). Our goal was to study the characteristics of analgesic reactions of animals under the influence of different wavelength ranges of halogen and fullerene light and to identify the contribution of fullerene component in reducing inflammatory pain depending on the spectrum, power density and illuminance. Methods: The experiments were performed on mice with an artificially created locus of inflammatory somatic pain (formalin test). The light source was Bioptron-MedAll device equipped with a halogen lamp. In separate series (80 animals in total) we studied the effect of light transmitted through fullerene filters (based on CR39 or PMMA) on the pain intensity and non-painful behavioral reactions when it was applied to the pain locus or to the AP E-36. Analgesic effects were compared with data obtained under the action of polychromatic (480+ nm and 320+ nm) light or 3 placebo light options, taking into account physical characteristics of the filters. Results: It was found that fullerene light gave the best analgesic effect regardless of the type of filter substance (PMMA or CR39). When fullerene light (CR39 filter) was applied to the pain locus, analgesia was 34.3%. An analgesic reaction was also detected during its action on the AP E-36, 32.6%, while the halogen polychromatic light was weaker (16.2%). The light passing through the fullerene+PMMA filter when applied to the pain locus alleviated pain by 29.8%. Polychromatic light application produced analgesia of 480+ nm was 23.2%, and 320+ nm, 14.4%. All placebo variants were less effective, although analgesia also occurred: 25.3% (CR39 fullerene free), 24.9% (PMMA fullerene free), and 27.7% (color copy of fullerene filter spectrum). The biological effectiveness of the studied light variants, estimated by the intensity of pain syndrome, correlated mostly with the power density and the wavelength range of light. Fullerene filters convert PL almost in the same way, with a similar analgesic result, but the biological effect of fullerene+CR39 light is more noticeable. Conclusion: Fullerene modified light, compared to halogen, has the greatest analgesic efficacy against formalin-induced pain. This result is achieved by the sorption of frequencies of the blue part of the spectrum by fullerene and depression of the power of the visible spectrum as a whole.

Keywords: Polarized polychromatic light, Bioptron-MedAll device, pain, formalin test, analgesia, fullerene, fullerene modified light.

1. Introduction

Currently, applications of low-intensity monochromatic laser light of various wavelength ranges from visible to infrared are successfully used in therapeutic practice [1-5]. However, due to their spectral narrowness, not all resonating biological structures are capable of an adequate reaction. Devices emitting polychromatic polarized light (PL) have found even wider application, among which the BIOPTRON devices (Switzerland) are most known. Studies of this variant of light have shown that it has pronounced biological activity, and this property can be successfully used in medical practice [6, 7].

Our previous studies on a model of inflammatory somatic pain in animals (formalin test) showed that when applied to a pain locus or acupuncture point

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(AP), low-intensity PL significantly relieves the pain [8-16]. With equal exposure, the effect depended significantly on the wavelength range of light radiation. The use of monochromatic filters that modified polychromatic light allowed us to obtain red, orange, yellow, green, indigo, blue, and violet ranges. For each of them, the fact of the analgesic effect of PL was experimentally established, which characterized the presence of a constantly acting factor, regardless of the wave range. On the other hand, additional effects have been discovered that depend on the physical specificity of definite ranges. Thus, “warm” colors, in particular red, more effectively suppress the inflammatory pain response compared to “cold” ones [15, 16]. The greatest analgesic effect had the red light. On the other hand, short-wavelength ranges (“cold”) proved to be more effective as bactericidal agents [17].

In recent years, new materials have appeared that are capable of changing the properties of light passing through them at the nanoscale. Among them, a molecular carbon—fullerene, discovered at the end of the 20th century, is of particular interest [18]. One of the properties of materials containing fullerene is the ability to influence the luminous flux. According to the hypothesis of Koruga [19], this effect is manifested in the change of photons direction from linearly polarized to toroidal. Accordingly, this light was called Bioptron®QuantumHyperlight® (Hyperpolarized light). Our studies showed that PL, which received additional properties due to modification by fullerene molecules (0.33% in PMMA), when acting on the locus of pain or on AP E-36 reliably relieves pain and has a significant sedative effect [20, 21]. In addition, having analyzed the results, it was revealed that in the development of the described changes, changes in the spectrum of the light transmitted through the fullerene layer may be significant.

Our goal was to study characteristics of the analgesic reactions of animals under the influence of different wavelength ranges of halogen and fullerene light and to identify contribution of the fullerene component in reducing inflammatory pain depending on the spectrum, power density, illuminance and filter design technology.

2. Materials and Methods

2.1 Animals

The experiments were performed on adult white male mice weighing 27-32 g. All experiments were carried out in accordance with the ethical recommendations of the International Pain Association. The animals were kept in the vivarium of Bogomoletz Institute of Physiology, NAS of Ukraine (Kyiv), in conditions of controlled temperature (18-200 °C) and 12-hour light day. All animals had free access to water and food (special granulated food). The day before the experiment, the mice were placed one by one in plastic cage and adapted to the experimental conditions. To reduce the effect of circadian rhythms on the nociceptive sensitivity of animals [22], all experiments were conducted at the same time of the light part of the day (from 10 to 13 h). Each mouse was used only in one experiment and at the end it was put to sleep by a lethal dose of urethane (intraperitoneally).

2.2 Creation of the Locus of Pain

The formalin test, which is standardly used to determine the analgesic efficacy of analgesics or physical factors, was chosen as an experimental model for quantitative assessment of pain intensity [23-25]. The pain was created by subcutaneous injection of a 5% formalin solution (10 μL/10 g of body weight) in the dorsal surface of the foot of the left hind limb. The locus of inflammation was a source of pain for several hours, but it was especially noticeable in the first 60 min. The intensity of pain was judged by the frequency and duration of the licking cycles of the diseased paw.
As it is known, the reaction to formalin injection consists of two phases [23, 24]. The first lasts no more than 10 min (acute pain) and the second—long phase (tonic pain caused by inflammation in the injection area). Since in our experiments, immediately after injection of a formalin solution, a 10-min application of light was made, during which the animal was in a special chamber partially limiting motor activity, we could not observe the first phase of the pain reaction. In the future, we will only talk about the second—the tonic phase of pain.

2.3 Applications of Polarized Light

Immediately after injection of the formalin solution into the paw, the mouse was placed in a plastic tube chamber with openings for air access. The hind left foot, in which formalin was injected, was brought out of the chamber through a special hole and gently held for 10 min by the hand of the experimenter. During these 10 min, PL was applied to the locus of pain or to the analgesic AP E-36. The animal of the control group was also in the chamber for 10 min with an elongated paw, but without light application (imitation of a light session). Each experimental group contained 10-12 animals, the control group (placebo-0)—20 animals.

The source of a continuous stream of polychromatic halogen light was the Bioptron-MedAll device, which created PL with a light power density of 40 mW/cm² and higher depending on the distance. To reduce the diameter of the light spot, a lightproof nozzle with a 5 mm hole was used. The distance from the glass of the filter to the skin surface was 5 cm, continuous exposure was 10 min.

We studied (Fig. 1) the analgesic efficacy of two variants of halogen-fullerene light (550-3,400 nm): transmitted through a flat filter made of polymethylmethacrylate (PMMA) containing 0.33‰ fullerene, and through a concave filter made from allyldiglycol carbonate (CR39) on the surface of which fullerene with a protective layer was applied. The results were compared with the effects of polychromatic light with a wavelength range from 320 nm (near UV) and 480 nm (without UV) up to 3,400 nm. To understand the role of the components generating fullerene light, we evaluated PL modifications created by passing halogen light through CR39 and PMMA filters that do not contain fullerene (placebo-1 and 2), and a flat filter that creates a color copy of fullerene filter spectrum light (placebo-3).

2.4 Investigations of Some Physical Parameters of the Light of the Bioptron-MedAll Device

To reveal the dependence of fullerene-halogen light efficiency on its physical characteristics, the following were measured: light power density, illuminance, and wavelength range. For these purposes, we used the appropriate certified equipment: OPHIR Nova II device (power density measurements), Lightmeter 401025 (illuminance), spectrometer Solar Laser Systems S-100 and Blu-Wave Vis-25 StellarNet Inc. (wave range).

2.5 Statistical Analysis

The data were processed statistically. With the help of a special computer program, we calculated the duration of pain (licking) and non-painful (slipping, washing, running, eating) behavioral reactions for every consecutive 10-min interval of time and for the entire observation period (60 min). Experimental data are presented as mean ± SEM. The reliability of the difference between the groups was determined by the Student’s test (t-test).

3. Results

3.1. Influence on Somatic Pain of the Halogen Light Transmitted through Afullerene+CR39 Filter when Applied to a Pain Locus or Analgesic AP E-36

Studies have shown that in animals that received application of fullerene+CR39 (550+ nm) PL on the locus of pain or on AP E-36, the pain was weaker than in animals that did not receive a light session (Fig. 2).
This is seen when comparing the curves showing the dynamics of the development of the pain process, and the total values. In general, during 60 min of observation after the application of fullerene light, the pain response was 359.9 s (locus of pain) and 369.3 s (AP E-36) versus 547.9 s in the control, which is 65.7% and 67.4% of the control value (Table 1). The difference is statistically significant ($p<0.05$). Analgesia was 34.3% and 32.6%.

The effect of fullerene light (550+ nm) on the locus of pain or on the analgesic AP E-36 not only weakened the formalin-induced somatic pain response, but also influenced non-painful behavioral responses of animals (Table 1). In the group receiving fullerene + CR39 PL, all 4 recorded non-painful reactions differed from the control. The most significant increase in sleep duration was 1.6 times, and food intake—2 times (Fig. 3).

Thus, application of a fullerene+CR39 PL to the pain locus or to analgesic AP E-36 weakens formalin-induced somatic pain. This is evidenced by both a decrease in the duration of the pain reaction, and an increase in the duration of sleep and food intake. The effect of exposure of AP E-36 on a CR39 fullerene light did not differ significantly from a similar effect to the pain locus (Table 1).
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Fig. 2  Dynamics (A) and total values (B) of the pain reaction duration before and after a 10-min of fullerene+CR39 (550+ nm) polarized light application in comparison with the control.
1—control series (placebo-0, without light application); 2—application of fullerene light to the locus of pain; 3—application of fullerene light to acupuncture point E-36.
The numbers above the columns are the group average duration of the pain reaction (s) for 60 min of observation. Significance of differences with the control: ** $p<0.05$.

Fig. 3  Dynamics (A) and total value (B) of sleep duration after a 10-min application of the fullerene+CR39 (550+ nm) PL to the pain locus or AP E-36 in comparison with the control.
1—control series (placebo-0, without light application); 2—application of fullerene light to the pain locus; 3—application of fullerene light to acupuncture point E-36.
Significance of differences with the control: * $p<0.5$. Designations as in Fig. 2.
The data obtained after application of PL to the pain locus, transformed by fullerene + PMMA filter (550+ nm), are summarized in Table 2 and Figs. 4 and 5. For comparison, it is shown the results were obtained under similar conditions in control animals (placebo-0/without light).

Comparing the average values of the recorded reactions in the group where the animals received a session of PL passing through the fullerene+PMMA filter, and in the control group (without light application), differences were found for all behavioral reactions except running. The two groups were most noticeably distinguished by the duration of pain and sleep. The significance level of the difference is high ($p<0.05$). Below we provide graphs illustrating the dynamics and total values of pain and non-painful reactions in the experimental and control groups.

The data obtained indicate a significant decrease in the intensity of pain in the group subjected to application of the PL transmitted through the fullerene+PMMA filter to the pain locus, compared with the control (without light application). The pain response was shortened 1.4 times. In the control group the pain was 547.9 s, and in the experimental group it was 384.5 s (70.2% of the control). The significance level of the difference: $p<0.05$.

Non-painful reactions under the influence of light also changed. In particular, noticeable differences were observed in the duration of sleep. It increased 2.2 times. In the group receiving the fullerene light session, the mice slept on average 1,076 s, against 492.3 s in the control group. The difference is significant ($p<0.05$). The most pronounced changes were observed in the second 30-min observation period, which significantly increased (5 times compared with the control group) the duration of food intake (Table 2). Increase in the duration of sleeping and eating also indicates weakening of pain under the action of fullerene light.

3.2 Effect on Somatic Pain of Halogen Light Transmitted through a Fullerene + PMMA Filter when Applied to a Pain Locus

- **Table 1** Duration (Mean±S.E.M.) of painful and non-painful behavioral reactions (s and % of control) for 60 min of observation in control animals (without light application, $n=20$) and with application of fullerene+CR39 PL (550+ nm) to the pain locus ($n=10$) or to AP E-36 ($n=10$).

| Groups                  | Duration of painful and none-painful reactions (s/60 min) |
|-------------------------|-----------------------------------------------------------|
|                         | Pain            | Sleeping        | Washing         | Running         | Eating          |
| Control (Placebo-0/without light) | 547.9 ± 52.02  | 492.25 ± 100.16 | 210.15 ± 26.31  | 103.35 ± 26.72  | 7.55 ± 3.98    |
|                         | 100% ± 9.49%   | 100% ± 20.35%  | 100% ± 12.52%   | 100% ± 25.85%   | (5 of 20)       |
| Fullerene +CR39 (pain locus) | 359.9 ± 48.53** | 793.1 ± 176.02* | 262.1 ± 55.96*  | 36.5 ± 6.58**   | 45.8 ± 42.47*  |
|                         | 65.69% ± 8.86% | 161.12% ± 35.7%| 124.72% ± 11%   | 35.32% ± 6.37%  | (2 of 10)       |
| Fullerene+CR39 (AP E-36) | 369.3 ± 59.86**| 966.7 ± 211.21*| 186.4 ± 24.95   | 82.3 ± 30.22*   | 29.2 ± 17.59*  |
|                         | 67.4% ± 10.93% | 196.38% ± 42.9%| 88.7% ± 11.87%  | 79.63% ± 29.24% | (3 of 10)       |

The top line is the reaction duration (s). The bottom line is the reaction duration, % of the control taken as 100%. In the column “Eating” in parentheses it indicated the number of animals that ate food, of the total number of animals in the group. Significance of differences with control: * $p<0.5$; ** $p<0.05$.

- **Table 2** Duration (Mean±S.E.M.) of painful and non-painful behavioral reactions (s and % of control) for 60 min of observation in control animals (without light application, $n=20$) and with application of fullerene+PMMA PL (550+ nm) to the pain locus ($n=10$).

| Groups                  | Duration of painful and none-painful reactions (s/60 min) |
|-------------------------|-----------------------------------------------------------|
|                         | Pain            | Sleeping        | Washing         | Running         | Eating          |
| Control (placebo-0/without light) | 547.9 ± 52.02  | 492.25 ± 100.16 | 210.15 ± 26.31  | 103.35 ± 26.72  | 7.55 ± 3.98    |
|                         | 100% ± 9.49%   | 100% ± 20.35%  | 100% ± 12.52%   | 100% ± 25.85%   | (5 of 20)       |
| Fullerene +PMMA         | 384.5 ± 34.9** | 1076 ± 220.7** | 178.1 ± 30.5*   | 77.8 ± 28.8     | 38.3 ± 35.8*   |
|                         | 70.18% ± 6.37% | 218.59% ± 44.83%| 84.75% ± 14.49%| 75.28% ± 27.91%| (3 of 10)       |

Designations as in Table 1. Significance of differences with control: * $p<0.5$; ** $p<0.05$. 

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3.3 Comparison of the Analgesic Effects of Fullerene Light, with the Effects of Polychromatic Light without UV or Pure Halogen Light (with UV)

The data obtained under the conditions of application of fullerene light (550+ nm) were compared with the results after the application of polychromatic light (480+ nm) and halogen light without UV (320+ nm) (Table 3).
Table 3  Duration of the painful behavioral reaction (s and % of control) for 60 min of observation in animals after application of fullerene light (CR39 and PMMA), polychromatic light without UV and halogen with UV light in animals after application to the pain locus or to AP E-36 in comparison with the control group (without light).

| Groups                  | Pain locus     | AP E-36       |
|-------------------------|----------------|---------------|
| CR39+Fullerene filter (550+ nm) | 359.9 ± 48.53**  | 369.3 ± 59.86** |
| PMMA+Fullerene filter (550+ nm) | 65.7% ± 8.86%    | 67.4% ± 10.9%     |
| Polychrome filter (480+ nm)        | 384.5 ± 34.92**  | -             |
| Without any filters (320+ nm)      | 70.2% ± 6.38%   | -             |
| Control (placebo-0/without light)  | 420.7 ± 69.1*   | 458.9 ± 49.8*   |

The top line is the reaction duration(s). The bottom line is the reaction duration, % of the control taken as 100%. Significance of differences with control: * p <0.5; ** p <0.05.

Fig. 6  Comparison of analgesic effects of the PL, modified with fullerene (CR39 or PMMA 550+ nm) or polychromatic (480+ nm) filters, as well as without the filter (320+ nm), application to the pain locus and to analgesic AP E-36.
Significance of differences with the control: * p<0.5; ** p<0.05.

Table 3 shows that, of all the types of light used, its fullerene variant (both after CR39 and PMMA filter) turned out to be the most effective in relation to pain suppression. Analgesia from the use of fullerene light was greater than from polychromatic light without UV and halogen with UV (Fig. 6). In case of
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fullerene+CR39 light application on the pain locus, analgesia was 34.3% and on AP E-36, 32.6%. The light passing through the fullerene+PMMA filter (550+ nm), with a 10-min application to the pain locus, provided a reduction in pain duration by 29.8%. The analgesic effect of polychromatic light application (480 nm +) was 23.2% (the locus of pain) and 16.2% (AP E-36). In this case, halogen light without a light filter (320+ nm) weakened the pain by 14.4% (pain locus) of the control value, taken as 100%. Therefore, fullerene enhanced the analgesic effect of polarized light.

3.4 Effect of Halogen Light Modified with Placebo Filters on Somatic Pain when Applied to a Pain Locus or Analgesic AP E-36

To test the contribution of fullerene to the above-described effect of pain suppression by light, we conducted experiments using a similar methodological scheme with three placebo filters. Placebo-1 filter was made of CR39, but did not contain fullerene, Placebo-2 filter of PMMA also did not contain fullerene. Placebo-3 filter also did not contain fullerene, but its wave spectrum was similar to fullerene. All other experimental conditions and the light source (Biptron-MedAll device) did not differ from those described above.

3.4.1 Effect of the Halogen Light Modified with a CR39 Filter without Fullerene (Placebo-1) on the Somatic Pain

We performed experiments on 10 animals with a locus of somatic pain exposed to PL from a Biptron-MedAll device with a CR39 placebo-1 filter (without fullerene coating). The data obtained were compared with similar data in animals without exposure to light radiation (control series) and with the results obtained using the light transmitted through a CR39 fullerene covered filter (Fig. 7, Table 4).

It was found that in the group, where the applied light passed through a placebo-1 filter (pain locus), the pain response was somewhat shorter (409.4 s) than in the control group (547.9 s). The significance level of the difference was very low (p<0.5). The analgesic effect of using placebo-1 light was 25.3% (locus of pain)
and 13.6% (AP E-36). As our previous studies showed, the effect of the use of fullerene light was higher. When fullerene light was applied to the pain locus, analgesia was 34.3%, and on AP E-36, 32.6%.

3.4.2 Effect of the Halogen Light Modified with a PMMA Filter without Fullerene (Placebo-2) on the Somatic Pain

The aim of this series was to establish the dynamics of pain (licking the pain locus) and non-painful (sleeping, running, washing, eating) behavioral reactions after exposure of the pain locus to PL transmitted through a PMMA filter that does not contain fullerene (placebo-2), and compare the result with the effect of fullerene+PMMA light. The results are summarized in Table 5 and in Fig. 8.

Compared to the control group, in animals receiving a 10-min application of PMMA light, the pain response was slightly weaker. However, these differences only after 50 min from the start of observation reached a high degree of reliability (p<0.05). The total duration of the pain response in the control group (547.9 s) and in the placebo-2 group (411.7 s) did not differ significantly (p<0.5). Analgesia was 24.9%.

Non-painful reactions in the placebo-2 group did not differ significantly from the control. The duration of sleep in this (540.1 s) and control (492.3 s) groups is almost the same. In contrast, the light passing through the PMMA filter with fullerene increased the duration of sleep by 2.2 times (1,076 s). Thus, in contrast to fullerene+PMMA light, application of placebo-2 light to the pain locus does not significantly change both pain and non-painful behavioral reactions in animals compared with the control.

3.4.3 Effect of the Halogen Light Modified with a Placebo-3 Color Filter with a Spectrum Similar to Fullerene on the Somatic Pain

In this series, it was determined whether there is a difference in the dynamics of pain and non-painful behavioral reactions after exposure to a pain locus with light of fullerene and non-fullerene origin with a similar spectral composition. When comparing the average values of the recorded reactions in the group where the animals received a session of PL passing through the placebo-3 filter, and in the control group (without light application), differences were found in all behavioral reactions except washing. Below we present graphs (Fig. 9) illustrating the dynamics and total values of pain and non-painful reactions in the experimental (placebo-3) and control (placebo-0/without light) groups, compared with data obtained after the action of fullerene+PMMA light.

It was found that in the group, where the Bioptron-MedAll light was applied through placebo-3 filter (the same color as fullerene filter, but fullerene-free), the pain response was somewhat shorter (396±73.1s) than in the control group (547.9±52 s). It amounted 72.3% of the control. Analgesia was 27.7%. As our previous studies showed, under the influence of light passing through a flat fullerene filter, the duration

Table 4  Duration (Mean±S.E.M.) of pain and non-painful behavioral reactions (s) for 60 min of observation in animals with a locus of tonic pain receiving a 10-min session of fullerene+CR39 light on the locus of pain and placebo-1 light in comparison with the control group (placebo-0, without light application).

| Groups                          | Duration of painful and none-painful reactions(s/60 min) |
|--------------------------------|---------------------------------------------------------|
|                                | Pain          | Sleeping       | Washing         | Running          | Eating          |
| Control (placebo-0/without light) | 384.5±34.9**  | 1076±220.7**  | 178.1±30.5*    | 77.8±28.8       | 38.3±35.8*     |
| CR39/fullerene free(Placebo-1)   | 409.4±52.3*   | 706.9±195.1*  | 282±57.6*     | 59.7±13.2*      | 19.1±11.5*    |
| Fullerene+CR39                   | 359.9±48.53** | 793.1±176*    | 262.1±56*     | 36.5±6.6**      | 45.8±42.5*    |

Significance of differences with control: * p<0.5; ** p<0.05.

Non-painful reactions under the influence of placebo-1 light (CR39), unlike fullerene+CR39 light, changed slightly. Significant differences were noted only in reactions such as sleeping and running (p<0.5). The use of fullerene+CR39 light with a high degree of reliability (p<0.05) shortened the pain response and increased the duration of sleep by 1.6 (pain locus) and 2 times (AP E-36).
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Table 5  Power density of Bioptron-MedAll device light depending on filter properties.

| Filter                          | Power density (mW/cm²) | Power density depression coefficient |
|--------------------------------|------------------------|-------------------------------------|
| Without filter                 | 125.7                  | 1.0                                 |
| Fullerene (0.33‰)+PMMA flat filter | 90.9                  | 0.723                               |
| Color copy of fullerene filter (placebo-3) | 92.4                  | 0.738                               |

Fig. 8  Dynamics (A) and total duration (B) of the pain response after 10 min of the placebo-2 light application to the pain locus in comparison with fullerene+PMMA light and with the control.

1—control series (placebo-0, without light application); 2—application of fullerene+PMMA light; 3—application of the placebo-2 (PMMA without fullerene) light.

Significance of differences with the control: * p<0.5; ** p<0.05. Designations as in Fig. 2.

Fig. 9  Dynamics (A) and the total value (B) of the pain response duration after 10 min of the placebo-3 application to the pain locus in comparison with fullerene+PMMA light and with the control.

1—control (placebo-0 without light application); 2—application of fullerene + PMMA light; 3—placebo-3 application (light with a spectrum similar to fullerene).

Significance of differences with the control: * p<0.5; ** p<0.05. Designations as in Fig. 2.
of pain decreased to 384.5 ± 34.9 s (70.2% of control). Thus, fullerene light was somewhat more efficient than light passing through a similar filter, but without fullerene. However, the difference between these groups is not statistically significant.

Non-painful reactions with placebo-3 light also changed. The duration of sleeping, running and nutrition in the experimental group increased compared with the control (without light application). In the experimental group, mice slept an average of 665.7 s, versus 492.3 s in the control group. Sleep duration increased by 35.2% of the control value. Mice were more mobile. Running duration increased by 42.4% (147.2 s versus 103.4 s). If we compare the eating behavior of animals, then in the experimental group 50% of the animals were eating (6 of 12), and only 25% in the control group (5 of 20). An increase in the duration of sleeping, running and eating, along with a decrease in the duration of the pain reaction, indicates weakening of pain under the influence of light passing through the placebo-3 filter.

3.5 Contribution of the Individual Physical Parameters of Light to Its Biological Effectiveness

Modification of light by various filters allows isolating its individual components and thus revealing the role of each of them (wave range, power density, illumination, polarization, etc.). Of course, other experimental conditions should remain unchanged. This is the approach we used to implement the research goal.

The values of the light power density of the Bioptron-MedAll device with the identical wavelength range of the filters are shown in Table 5. The difference was only in the presence or absence of the fullerene in filter material. The measurements revealed that the power density of the light passing through the fullerene filter and placebo-3 filter was almost the same (90.9 and 92.4 mW/cm²). This indicates the proximity of these two filters in terms of efficiency (Fig. 9). If we neglect the insignificant differences due to the fact that the object of study, although it was standardized, is live (mouse) with individual variations, then we can talk about the identity of the response, with close values of the power density and the wavelength range of light.

Analysis of these data allowed us to obtain an additional practical result: a fullerene filter with a fullerene concentration of 0.33‰ reduces the light load on biological tissues by 28%. This coefficient of light load depression can be considered as an indicator of the efficiency of the light-shielding effect of fullerene filters, which are currently used, on the one hand, and as a factor weakening a possible biological response, on the other.

Despite the fact that the power density of light of the Bioptron-MedAll device without a filter was the highest compared to fullerene or placebo-3 light, the analgesic effect was minimal in this case. This means that there is no direct proportional dependence of the biological effect on the power of light in the studied power range. This result can be viewed from the perspective of biological antagonism of the red and blue parts of visible light. The “pure” light of a halogen lamp has an extended range of the blue part (from 320 nm and higher), while the range of fullerene light begins with only 450 nm, and its biological effectiveness began with a conditional threshold for a biological reaction to light (20 mW/cm²) — from 520 nm (Fig. 10). The wave shift, based on spectral data, was 130 nm, and as to biological efficiency — 70 nm.

Analysis of the spectra shown in Fig. 10, revealed a gradient between the averaged sections on the spectrogram plateau, which is stably maintained at all wave frequencies. This means that light load depression caused by an admixture of fullerene in the filter is a constant factor.

We also examined the possible effects on the biological efficiency of different illuminances of the application area with different filters. We evaluated the illuminance (lux) produced by the Bioptron-MedAll PL without a filter, with fullerene and with placebo-3 filters.
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Fig. 10 Comparison of the wave light spectra of a halogen lamp of the Bioptron-MedAll device before and after its modification with fullerene.

1—light spectrum of a halogen lamp of Bioptron-MedAll device; 2—light spectrum of a halogen lamp of Bioptron-MedAll device equipped with a PMMA-based fullerene filter; 3—averaged plateau of the spectrum of a halogen lamp; 4—averaged plateau of the spectrum of the fullerene light; 5—gradient between the averaged sections of the spectra; 6—a reference line showing the starting point of the halogen lamp light spectrogram; 7—point of the beginning of the light spectrum of the halogen lamp of the Bioptron-MedAll device (located outside the sensitivity zone of the spectrometer Blue-Wave Vis-25 StellarNet Inc); 8—a reference line showing the starting point of the fullerene light spectrogram; 9—gradient between the starting points of the spectra; 10—level of the conditional threshold of the biological response to light (20 mW/cm²); 11—area of the spectrum from which the biological reaction to halogen light begins; 12—area of the spectrum from which a biological reaction to fullerene light begins; 13—fullerene-induced displacement of the zone of the biological reaction beginning.

Spectra were recorded by spectrometer Blue-Wave Vis-25 StellarNet Inc. with a sensitivity range within 300-1,000 nm.
Our studies did not reveal a direct dependence of the biological effect of PL on the illuminance of the skin area (in the range of the studied values). Despite the fact that the light passing through the fullerene filter had the lowest illuminance coefficient (0.57), it turned out to be the most effective. Comparison of the pain response to pure halogen light or a light-simulator (placebo-3), which had the best values of the luminance factor (1.0 and 0.84), as shown above, did not reveal the advantages of such light options.

It should be noted that the light of the Bioptron-MedAll device as a whole made a noticeable heating of the skin surface to which it was directed. After 10 min of standard exposure, its temperature increased by 8°C (from 27°C to 35°C). The thermal factor can affect the biological efficiency of light. Such a temperature difference can be clinically useful, since better conditions are created for the activation of microcirculation. However, for a physiological experiment, it can cause the leveling of differences in the efficiency of different filters, which possibly explains the low reliability of difference between the groups.

4. Discussion

With the advent of a new light modifier (fullerene), it became necessary to clarify the existence of its specificity in the biological effect. Several variants of light that are close in effectiveness, for example, polychromatic, fullerene, etc., fall into hands of a doctor or user. Therefore, a comparative analysis of the effects turned out to be important. On the one hand, it expands the therapeutic opportunities of light therapy, on the other hand, it provides the possibility of a more accurate assessment of the effects and ranking of equipment.

Our studies were aimed at identifying and delimiting the contribution of factors very close in their final biological effect. This required experimental technology that would be standardized not only in the framework of this work, but also in research over the previous 20 years [13, 20, 26, 27]. This made it possible to adequately compare new results with data from previous studies, which was necessary in each case. All this increased confidence in the reliability of the results.

If we talk about the role of the fullerene filter as a modifier of the basic halogen linearly PL, we can notice three types of properties of such light, which may have biological significance. This is elimination of the blue part of the polychromatic spectrum, general frequency depression over the entire wavelength range, and the effect of reformattting linear polarization (hyperpolarization and toroidality) of the green-red part of the spectrum. The first two factors are understandable from a comparison of spectrograms. They have experimental confirmation, including indicating the contribution of fullerene light to the biological response. At the same time, the question of the existence and role of transformation of the light polarization by fullerene still needs physical and experimental confirmation. The theoretical hypothesis looks very tempting [19], however, due to its representation only at the quantum level, it is still problematic to obtain experimental evidence of the role of hyperpolarization (polarization contribution of the fullerene component) at the physiological and clinical levels. Based on the current volume of physical indirect evidence of the presence of toroidal polarization, it is difficult to get rid of the idea of the dominant role of fullerene as a sorbent of certain frequencies and the power depressant of the entire visible spectrum. As a result, we are forced to talk about the polyetiological nature of the fullerene phenomenon (indicated by the dotted line so far) to the explanation of which we are much closer due to the use of various types of placebo. However, the “youth” of the hypothesis and the first data surrounding it allows us to hope for a positive stabilization in the professional environment in the near future.

This does not mean that the consumer properties of fullerene filters should suffer due to theoretical
discussions. Now we can confidently talk about the clinical potential of fullerene light, proven on pain models and for various methodological application schemes [20, 28, 29]. Research is being carried out in many directions, and there is a hope for appearance of knowledge clarifying or expanding the current state of the optical, physical, and biological properties of fullerene light. Experimental and clinical evidence has been obtained of the effectiveness of fullerene filters mounted in glasses (Tesla Hyperlight Eyewear® (THE®glasses) [30-33]. They were identified in the analysis of the biological effectiveness of the initially unpolarized fullerene light, which acted through an ocular analyzer. The advantage of this variant of light was activation of the default zones and improvement of the quality of sensorimotor reactions detected by electroencephalography. Positive changes under the influence of fullerene light also consisted in improving mental performance (speed of processing information, switching attention during correction work, etc.). Methodologically, in these studies, a placebo-1-2 level was achieved, which allowed obtaining adequate reliability of the results.

The experimental data described above indicate the presence of the analgesic effect of fullerene PL when it is applied to the locus of pain or AP E-36. This effect is noticeable both for the light passing through the CR39 filter (concave filter with fullerene coating) and through the PMMA filter (flat filter, which contained 0.33‰ fullerene). If we compare the results of the action of different types of the investigated light, we can see that analgesia from the use of fullerene light was greater than from polychromatic (with or without the near UV range). So, when applying to the locus of pain, any version of fullerene light acted on pain: after the CR39 filter, it reduced pain by 34.3% and after the PMMA filter by 29.8%. Under the same conditions, polychromatic light without UV alleviated pain by 23.2%, and light with UV by 14.4% compared with the control. Consequently, the use of a fullerene filter enhances the analgesic effect of PL created by the Bioptron-MedAll device. These results are consistent with data obtained previously on a similar model using the BIOPTRON-Compact device with a fullerene filter [20]. Analgesia in this case was 43.5% (locus of pain) and 38.5% (AP E-36). Understanding of the general biological comparability of many syndromes and pathophysiological mechanisms in mammals and the results of our experiments on laboratory animals suggest that fullerene filters can enhance the biological effectiveness of polarized polychromatic light in humans as well as in domestic animals.

Let us note that confirmation of the presence of the reaction we proved earlier [8, 10, 13, 34] from the non-contact action of light (polarized polychromatic, fullerene) when it is applied to the locus of inflammatory pain (local effect) and to the acupuncture point (systemic effect) is again obtained. The acupuncture point E-36 (Zu-San-Lee) is considered analgesic with a wide range of additional effects, in particular, general tonic, immune-correction, etc. These data indicate that fullerene+CR39 PL causes not only an anti-inflammatory effect. The effect on the acupuncture point E-36 indicates the possibility of obtaining a systemic reaction, in this case an analgesic [35, 36]. We also obtained similar data for the action of halogen poly- and mono-chromatic light on AP [11, 28, 29]. We previously proved the presence of electromagnetic signal transport to the level of neurons in the central nervous system and activation of the opioidergic nociceptive system [25, 37]. This gives clinicians reason to use fullerene light puncture in practice, at least to relieve somatic pain. On this basis, effective schemes for the correction of pain syndromes have been developed [27, 29]. Similarly, it seems possible to obtain therapeutic effects when using fullerene light.

To understand the role of fullerene, and not the complex of fullerene+PMMA or fullerene+CR39, we consider the results obtained with placebo filters (3 options). Two of these filters were made of CR39 or PMMA, but without fullerene (placebo-1 and 2), and
the third (placebo-3) was identical in terms of the wave spectrum of fullerene+PMMA filter. It was established that all three placebo variants of the light had a biological effect, but it was less than fullerene light. When placebo-1 light was applied to the locus of pain, analgesia was 25.3%, placebo-2 light, 24.9%, and placebo-3, 27.7%. Thus, it was found that fullerene light creates an additional antinociceptive effect. Its value is moderate, although statistically confirmed.

The effectiveness of fullerene-halogen light, which we evaluated on the basis of direct data obtained on the formalin pain model, is likely to be uneven depending on its physical characteristics. The above data showed that the analgesic efficacy of light, assessed by intensity of the pain syndrome, is more correlated with the power density than with the illuminance values.

Even more important, apparently, is the wavelength range of light. The greatest biological effect is caused by the light passing through the fullerene filter. Other types of polychromatic light had a less pronounced analgesic effect. This belongs to ranges starting from 320 and 480 nm. The range of fullerene light begins with values of about 550 nm, but the threshold of the biological reaction is overcome after 600 nm. This is due to the fact that the fullerene filter filler blocks the blue part of the spectrum and reduces the power density of the spectrogram as a whole. In this case, the fullerene filter weakens the blue part of the spectrum to a greater extent than its main component—PMMA. Such a structure of fullerene light (green-red) brings it closer to the range of the orange and red filters we studied earlier, produced by classical glass-blowing technology. And it was precisely these ranges that were most effective in relation to analgesia [29, 38].

The biological efficiency of the polarized light of the Bioptron MedAll device can be affected by the thermal factor. The temperature gradient 8°C was experimentally determined for it (from 27 to 35°C), although for the BIOPTRON-compact device equipped with a fullerene filter, it was 4.6°C [20]. Raising the temperature up to 35°C is not biologically critical, since this is below the threshold of response of pain receptors. The effect of thermo-puncture also requires higher temperatures, it is necessary to heat the skin up to 43°C. However, at a temperature of 35°C, non-painful thermal receptors are triggered [39], which may contribute to the analgesic effects of the Bioptron-MedAll device. The average maximum of the static warm fiber discharge is at 46°C (from 30 to 48°C). Heating the skin inevitably causes blood flow to the locus of pain, which contributes to faster absorption of the locus of inflammation and has an analgesic effect. We believe that the thermal effect on the skin, which exists for all types of filters, eliminates the difference between them. As a result of this, we have a low level of statistical difference between different experimental groups. All this indicates that the biological effect of the Bioptron-MedAll device in all the series described above was determined by the sum of the light and thermal effects.

For an additional factor that can affect the overall painkiller result we would also call the effect of microwave radiation. We previously discovered it in the radiation of the Bioptron-compact device [40]. It is experimentally proved, that in the spectrum of polarized polychromatic low-energy radiation with wavelengths 480-3,400 nm is present a microwave component. It is within 37 up to 53 GHz range, that is outside of visible light, power of microwave radiation—up to $4.6 \times 10^{13}$ W/cm². The power density of the BioptronMedAll device is higher than that of the Bioptron-compact, closer to the values recorded without a UV filter. This means that it would be unreasonable to exclude an additional biological effect. So, the main options for fullerene light are considered, which, using placebo, explained the role of almost all of its components, with the exception of the contribution of toroidal (hyper) polarization.

To analyze the data of the differences in the analgesic reactions after application of PL transmitted through a fullerene filter (Fig. 6) to the pain locus, we can notice one more circumstance that we have not yet
focused on. We compared biological responses to a standard stimulus in the form of a dosed subcutaneous injection of a formalin solution depending on the structure of the fullerene filter, the material of which (two options) could possibly modify the PL in different ways. In this case, the identity of almost all the variables of the final acting factor (light) was observed: the source of PL, the presence of fullerene, the spectral composition, the light power density close in magnitude, exposure, and experimental technology. The difference was only in the structure of filters: a flat monolithic of PMMA, into the substance of which fullerene powder was uniformly impregnated at a concentration of 0.33‰ (light power density 90.9 mW/cm² from a distance of 5 cm), and a concave CR39, on which, on both sides 2 layers of fullerene were applied with appropriate protection against damage (86.9 mW/cm² from a distance of 5 cm). It turned out that the most noticeable analgesic effect was created by PL modified with a concave filter fullerene+CR39. It attenuated the formalin-induced pain/inflammatory response by 34.3%, while light transmitted through the flat fullerene+PMMA filter by 29.8%. The difference between the two experimental groups characterized a stable trend, the reliability of which was p<0.5. This means that both filters convert PL approximately the same, but the biological effect of fullerene+CR39light is more noticeable.

The range of growth of the bioeffect that arose due to the modification of polarized light by fullerene turned out to be almost completely filled. At present, it is not yet possible at the current technical level to detect the presence of toroidal polarization and the distribution of quanta in the light flux, obeying the Fibonacci law. The key question, the answer to which is necessary for the further use of fullerene light in order to increase its biological effect, retains the receipt of physical and biophysical evidence of previously expressed hypothetical views and the identification of physiological and clinical reactions in response to each new biotropic factor.

5. Conclusions

The polychromatic polarized light of the BioptonMedAll device passing through a fullerene filter (regardless of its basis or structure—CR39 or PMMA) reliably attenuates the pain response caused by formalin. This effect can be achieved by applying light to both the locus of pain and AP E-36. Analgesia from the use of fullerene light is more noticeable than from polychromatic light without UV (480+ nm) or with UVA (320+ nm).

When applied to the pain locus, fullerene light weakened the inflammation pain by 34.3% (CR39 filter) and 29.8% (PMMA filter), polychromatic light by 23.2%, and light without filter by 14.4% compared with the control. When placebo light was applied to the pain locus, analgesia was 25.3% (CR39 fullerene free), 24.9% (PMMA fullerene free), and 27.7% (color copy of the fullerene filter). Thus, fullerene enhances the antinociceptive effect of PL, with a more noticeable correlation with the power density and wave range than with the illuminance values. This result is achieved by sorption of the frequencies of the blue part of the spectrum and depression of the power of the visible spectrum as a whole.

Acknowledgments

This study became possible due to support of Mr. Philip Zepter, President of the Zepter International, Ms. Diana Zepter, Chief Executive Officer of the Zepter International, Mr. Nedzad Sokoljak, Vice President of the Zepter International and Dr. Dr. Marcel W. Hüppi, General Manager BIOPTRON AG.

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