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Changes in chemiluminescence of whole blood of COPD patients treated with Hypoxen® and effects of C₆₀ fullerenes on blood chemiluminescence

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Summary

Background:

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease associated with reactive oxygen species (ROS) production. The aim of this study was to evaluate the effect of Hypoxen® treatment and the effect of HyFnC₆₀ on ROS production in patients' blood.

Material/Methods:

ROS production in blood was estimated using chemiluminescence (CL) measurement with CL-amplifiers: luminol (LM), LM + zymosan (ZM) or lucigenin (LC) in the presence or absence of hydrated fullerenes (HyFnC₆₀) added to blood in low concentrations.

Results:

In all the patients with COPD in remission phase with Hypoxen® prescription, the LM-dependent CL (LM-CL) with ZM and LC-enhanced CL (LC-CL) decreased after the treatment. Parameters of CL and effects of HyFnC₆₀ upon them depended on blood state. Addition of HyFnC₆₀ to blood decreased data scattering and helped to improve discrimination between different groups of patients. Using the discriminator analysis, we found the most important time-points in the kinetic curves of CL for classification of patients into groups (eg, COPD patients before and after treatment with Hypoxen®; patients' blood with different sensitivity to HyFnC₆₀ concentration).

Conclusions:

Monitoring of CL of non-diluted whole blood in COPD patients can be used for the estimation of the Hypoxen® efficiency in complex therapy. Addition of HyFnC₆₀ to blood increases sensitivity of the method.

key words:

blood • chemiluminescence • reactive oxygen species • chronic obstructive pulmonary disease • Hypoxen® • hydrated C₆₀ fullerenes

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BACKGROUND

The bronchopulmonary system depends on the oxygen balance in the whole body, so broncho-obstructive disease or injury to the lung parenchyma will inevitably lead to the development of more or less deep hypoxia, depending on the severity of the disease. One such disease is COPD, which is characterized by airflow limitation and prolonged chronic inflammation that violate the architectonics of the bronchi and lung parenchyma [1]. It is believed that some diseases, such as cardiovascular diseases, osteoporosis, cachexia, and anaemia, are systemic consequences of COPD [2,3].

The key mechanism of pathogenesis of COPD is the inflammation that occurs in response to exposure to toxic substances that leads to inflammation, which contributes to mucus secretion, fibrosis, and destruction of alveolar walls. In addition, genetic factors [4], the immune system, oxidative stress, and abnormal expression of reparative processes contribute to the development and maintenance of COPD.

Currently, the role of oxidative stress is regarded as an important additional factor contributing to the disease [5,6]. Under the conditions of hypoxia and inflammation, characteristic for COPD, different cells are involved in the generation of ROS, represented by superoxide anion radical, hydrogen peroxide, hydroxyl radical and other highly reactive chemical compounds. Reactions in which ROS appear may be monitored using sensitive luminometers if chemiluminescent probes, in particular LM or LC, are added to the reaction systems.

ROS are highly chemically reactive and need to be controlled to prevent damage. The system of control is generally known as the antioxidant system. In this study we used Hypoxen[®], which is the promising new antioxidant and anti-hypoxant that demonstrates remarkable ROS protective effect in patients with COPD and appears to have very few contradictions.

The aim of the current study was to compare the level of ROS in the undiluted whole blood of 3 groups of volunteers: patients with COPD before treatment with Hypoxen[®], patients with COPD after treatment with Hypoxen[®], and patients suffering from acute attack. A statistically significant decrease in ROS contents in blood was observed after Hypoxen[®] treatment of COPD. We also found that addition of HyFnC_{60} to whole blood generally alters the intensity of CL developing in blood, and also decreased data scattering between parallel probes.

MATERIAL AND METHODS

This was an open, nonrandomized, prospective study of the efficacy of Hypoxen[®], including patients with COPD (GOLD stages III and IV). Criteria of inclusion: age ≥ 40 years, smoking ≥ 20 packs/year, forced expiratory volume in 1 second (FEV1) $< 50\%$, FEV1/forced vital capacity (FVC) $< 70\%$ of predicted, stable COPD, no changes in treatment, and no exacerbations COPD. Therapy for COPD includes treatment with a β_2 -agonist and/or anti-cholinergic and/or inhalation corticosteroids.

The study was approved by the Hospital Medical Ethics Committee (Moscow City Clinical Hospital #23 *Medsantrud*), and all the patients gave informed consent.

Figure 1. Hypoxen structure.

The patients (60 persons) involved in the study took Hypoxen[®] 250 mg (750 mg b.d.) during 2 months. Hypoxen[®] therapy was added to the standard treatment of stable COPD.

Hypoxen[®] (*Polydihydroxyphenylthiosulfonate sodium*) (Figure 1).

The estimates of efficacy were based on spirometric assessment of lung function. Patients had a FEV1 and FVC by spirometer (Master Screen Pneumo, Erich Jaeger GmbH, Germany). Oxygen saturation (SaO_2) was estimated by pulse oximeter (Beijing Choice Electronic Tech Co., LtdJ., China). We used the COPD Assessment Test (CAT) for measuring the impact of COPD on patients (<http://www.CATestonline.org>). Lung function tests, SaO_2 , and CL were made the first and the last day of the study (2-months treatment with Hypoxen[®]).

The patients with acute attack of COPD were involved in the study as a comparison group. We compared the level of CL in the blood in the patients with stable COPD and exacerbation of COPD. The measurement was made only once in both groups of patients. The patients with exacerbation of COPD did not take Hypoxen[®]. Therapy of acute attack of COPD included antibacterial treatment and/or corticosteroids (i.v. or per os), mucoactive treatment (except for N-acetylcysteine), β_2 -agonist by nebulizer (Table 1).

Summary statistics are presented as proportions [with 95% confidence intervals (CI)] or means [with standard deviations (SD)]. The invariable associations between time period and variables were examined using 2-sample *t*-tests.

Blood of patients with COPD was obtained by venous puncture and was stabilized by heparin 0.1 ml (1000 IU)/10 ml of blood. Blood was obtained between 9.00 and 10.00 a. m. and was used exactly 3 hours after extravasion. During this period it was kept in plastic tubes at 20–25°C.

Whole undiluted blood CL was registered after addition to blood of either LC (a probe for superoxide radical anion) or LM (a probe for multiple ROS). The stock solution of LM was obtained by dissolving it in DMSO to a concentration of 0.02 M. The stock solution of LC was obtained by dissolving it in saline to a concentration of 0.02 M. Both were added to whole blood to a final concentration of 0.1 mM. In cases when respiratory burst was induced in blood by ZM, the latter was added to blood to make a final concentration of 0.5 mg/ml, 5 minutes after LM addition. Additives (10 μl each) were added to 0.2 ml of blood in 1.5 ml Eppendorf polyethylene test tubes.

Table 1. Characteristics of patients with COPD.

	Stable COPD	Exacerbation of COPD
No. of patients	60	20
Age, years	60.5±8.7	58.2±9.2
Smoking, packs/years	36.9±9.18	40.2±7.8
Therapy of LABA,% of patients	60	50
Therapy of ICS,% of patients	80	60
Dose of ICS (beclometasone), µg	648.9±163.2	726.2±124.2
Therapy of IA,% of patients	40	40
FEV1, L	1.32±0.53	0.62±0.43*
FEV1,% of predicted	39.6±14.1	25.6±9.6*
FEV1/FVC,% of predicted	53.9±10.85	44.2±9.5*
SaO ₂ ,%	94.8± 1.29	89.7±0.5*
CAT	19.3± 9.3	10.1±6.2*

* $p < 0.05$; LABA – inhaled long-acting β_2 -agonist; ICS – inhaled corticosteroid; IA – inhaled anticholinergics.

Initial solution HyFnC₆₀ (1 mM) was provided by G.V. Andrievsky. Serial decimal dilutions of HyFnC₆₀ were carried out in saline and each next dilution was stirred on a Vortex shaker for 5 seconds. Five µl HyFnC₆₀ dilutions were added to blood 1 min before adding of LM or LC. As a control, 5 µl of saline solution was added to blood instead of HyFnC₆₀.

CL from blood was registered using a single photon counter «Biotox-7a» (Russia) (Figure 2), equipped with a photomultiplier with a S-11 photocathode having a maximum response (sensitivity) in the wavelength range 400–500 nm, and a window 5 cm in diameter. The photomultiplier was deployed horizontally and registered flux of photons from the lateral surface of test tubes with blood. Kinetics of CL was registered from undiluted whole blood without or in the presence of HyFnC₆₀ for 5–15 min. The results were automatically fed to the computer in the ASCII format. CL intensity was expressed as the number of impulses per second. All the operations were performed at dim ambient illumination.

Processing of the results and calculations was performed using the software packages Microsoft® (USA) – Microsoft Office 2003 – Excel 2003; statistical multifunctional program Statistica V.5.0 and V.6.0. Discriminant analysis (DA), a section of multivariate statistical analysis, was used for the interpretation of intergroup differences, classification and analysis of the results obtained in the studied group of patients.

RESULTS

There were no differences in age, smoking and treatment characteristics between stable and exacerbation of COPD patients. The patients with exacerbation of COPD had lower FEV1, FEV1/FVC, SaO₂ and CAT than patients with stable COPD ($p < 0.05$).

We have not found any changes in FEV1 (L and% of predict.), FEV1/FVC (% of predict) and SaO₂ before and after taking Hypoxen® (Table 2).

Figure 2. Equipment for the registration of nondiluted whole blood CL (Biotox-7a).

The data (Figure 3) show decrease in breathlessness, increase in any activity at home, improvement in feeling of confidence and increase in energy in the patients after taking Hypoxen®. We did not find any influence on cough, mucus, feeling of tightness in the chest and sleeping ($p > 0.05$).

We found statistically significant decrease in total score of CAT after Hypoxen® therapy (18.3 ± 9.3 vs. 15.2 ± 9.0 [$p = 0.0002$]).

Dynamics of CL of whole venous blood of all patients was measured and analyzed. Dynamics of clinical symptoms in all patients with COPD during the treatment with Hypoxen® was also estimated. In each case, measurement of CL intensity was carried out on not less than 3 samples of undiluted blood of patients with COPD. Typical kinetics of CL in blood of 2 patients before treatment, after treatment with Hypoxen® with different CL intensity is presented in Figure 4, 5.

Table 2. Lung function measurements, results of SaO_2 and questionnaire data of CAT in the patients with stable COPD.

	Before taking Hypoxen®	After taking Hypoxen®
FEV1, L	1.32±0.53	1.37±0.43
FEV1,% of predicted	39.6±14.1	42.7±10.6
FEV1/FVC,% of predicted	53.9±10.85	56.2±11.2
SaO_2 , %	94.8±1.29	94.9±1.4
CAT	19.3±9.3	15.3±9.0*

* $p < 0.05$. **Figure 3.** Changes in clinical parameters of patients before Hypoxen® treatment and after it.

Parameters of blood CL depend both on the health status of patients, and properties of blood of a particular individual reflecting the immunity status of a patient. Therefore, the CL intensity of blood varied considerably from patient to patient. However, after treatment with Hypoxen®, CL intensity of blood was lower than that in patients prior to the application of Hypoxen®, as a rule, regardless of the intensity of CL (Figures 4, 5).

After treatment with Hypoxen® patient's P. and patient's R. FEV1 rose to 130 and 120 mm, respectively. Patient's P. SaO_2 increased from 95% to 97%. In contrast, patient's R. SaO_2 decreased from 95% to 93%. Both patients improved exercise tolerance from 2 to 3 points on the SAT Questionnaire. Addition of HyFnC_{60} to blood of COPD patients before and after Hypoxen® treatment altered LM-amplified CL-response to ZM and LC-CL (Figure 6). It is interesting that HyFnC_{60} in low (10^{-6} M) and especially ultra-low (10^{-19} M) concentrations drastically reduced LM- and LC-CL of blood after Hypoxen® treatment, amplifying the contrast between the groups of patients before and after treatment.

In many cases of patients suffering acute attack, both LM-dependent CL and especially LC-CL of blood were rather high. Addition of 10^{-6} M or 10^{-19} M of HyFnC_{60} to blood usually attenuated CL (Figure 7). It is interesting that HyFnC_{60} in concentrations 10^{-7} M and 10^{-11} M did not have any effect on blood CL.

Figure 4. (A) Luminol and luminol+Zymosan, and (B) lucigenin dependent CL (high intensity case) of nondiluted blood before and after Hypoxen® treatment (patient P.). 1 – before Hypoxen® treatment, 18.08.09; 2 – after Hypoxen® treatment, 06.10.09.

To apply DA to the all the experimental data, the averaged data of CL kinetics for all the patients were plotted on graphs. Figure 8 shows averaged kinetics for blood of patients in remission before treatment with Hypoxen® and after treatment, as well as the blood of patients in a state of acute attack, Figure 9 shows the kinetics of CL of blood of patients in the presence of 10^{-6} M of HyFnC_{60} (Figure 9A), and 10^{-19} M of HyFnC_{60} (Figure 9B). In these graphs we can see the difference between kinetics of CL of blood before and after treatment and in the state of acute attack, and the effects of HyFnC_{60} upon CL parameters.

Figure 5. (A) Luminol and luminol+Zymosan, and (B) lucigenin dependent CL (low intensity case) of nondiluted blood before and after Hypoxen® treatment (patient R.). 1 – before Hypoxen®, 23.10.09; 2 – after Hypoxen® treatment, 29.12.09.

We compared the intensity of CL at selected time-points (143 s, 260 s, 772 s, 804 s, 860 s, 907 s, 951 s, 1094 s, 1110 s, 1150 s) corresponding to the maximum difference in the intensity of CL between the groups, and applied DA for these time-points. The results of such data processing may be presented in the form of the coefficients of the classification function for each patient's group in tabular form.

The coefficients of the variables for the discriminant function were calculated using Statistica V.6.0. A linear classification function for patients from different groups was designed. A linear function:

$$d_{km} = \beta_0 + \beta_1 x_{1km} + \dots + \beta_p x_{pkm}, \quad m = 1, \dots, n, \quad k = 1, \dots, g$$

The function is called the canonical discriminant function with unknown coefficients β_i . Here d_{km} – the value of the discriminant function for the m -th object in the group k ; x_{pkm} – the value of the discriminant variable x_i for the m -th object in the group of k . From a geometric point of view, the discriminant functions define a hypersurface in p -dimensional space. In the particular case $p=2$ it is a straight line, and $p=3$ plane [7].

Thus, estimation of CL intensity of blood of all the tested patients before and after treatment allowed us to pick out time-points during the registration process of CL.

Figure 6. Effects of HyFnC₆₀ on Luminol and luminol+Zymosan- (A) and lucigenin- (B) dependent CL of nondiluted blood of COPD patient T. at HyFnC₆₀ action. 1 – before Hypoxen® treatment, 15.10.09, no HyFnC₆₀; 2 – 1 + HyFnC₆₀ (10⁻¹⁹ M); 3 – after Hypoxen® treatment, 22.12.09, no HyFnC₆₀; 4 – 3 + HyFnC₆₀ (10⁻⁶ M); 5 – 3 + HyFnC₆₀ (10⁻¹⁹ M).

For example, discrimination functions for groups of patients with COPD before (1), after (2) are the following:

$$D1 = -1.82 - 0.0042 * X_{143} + 0.0029 * X_{260} + \dots - 0.0043 * X_{1150},$$

$$D2 = -1.88 - 0.0011 * X_{143} + 0.00086 * X_{260} + \dots + 0.0074 * X_{1150},$$

where X_{143} , X_{260} , X_{1150} – CL intensity values in corresponding time points.

Discrimination functions for 3 groups of patients with COPD (before and after treatment with Hypoxen® and during the acute attack) were also calculated (Figure 10). Here the probability of discrimination between 3 groups was 54%. In the presence of 10⁻⁶ M or 10⁻¹⁹ M HyFnC₆₀ in blood, the probability of discrimination between 3 groups increased to 77% and 64%, respectively. Thus, addition of HyFnC₆₀ in low and ultra-low concentrations to blood of patients for the evaluation the parameters of LM, LM+ZM, and LC-CL helps to improve discrimination between the groups of COPD patients.

Application of these classification functions to the whole set of experimental data of CL measurement of whole blood of patients before and after Hypoxen® treatment

Figure 7. Effects of HyFnC₆₀ on Luminol and luminol+zymosan- (A) and lucigenin- (B) dependent chemiluminescence of nondiluted blood of COPD patient T-v. suffering acute attack. 1 – no HyFnC₆₀; 2 – HyFnC₆₀ (10⁻¹⁹ M); 3 – HyFnC₆₀ (10⁻⁶ M).

Figure 9. Changes in the dynamics of the average values of the intensity of whole blood CL in patients with COPD before (2) and after (3) therapy with Hypoxen® and during acute attack (1) in the presence of HyFnC₆₀: (A) – 10⁻⁶ M; (B) – 10⁻¹⁹ M.

Figure 8. Changes in the dynamics of the average values of the intensity of whole blood CL in 30 patients with COPD before (2) and after (3) therapy by Hypoxen® and during acute attack (1)

allowed us to evaluate the probability of inclusion of patients to a particular group, which is 72%. Taking into consideration that the whole number of patients whose blood was used for CL measurements was only 30, this probability is rather high. If CL measurement was performed on blood in the presence of 10⁻⁶ M or 10⁻¹⁹ M HyFnC₆₀, the

Figure 10. The measured parameters changed in a group of COPD patients before and after Hypoxen® treatment and at acute attack by 54% without HyFnC₆₀ (1st bar). In presence of HyFn (10⁻⁶ and 10⁻¹⁹ M) in blood of these patients – by 77 and 64% (2nd and 3rd bars). Kinetic CL parameters of COPD patients blood before and after Hypoxen® treatment can be discriminated with the probability of 72, 85 and 83% (4th, 5th and 6th bars respectively). The results of the 5th bar were obtained in presence of 10⁻⁶ M HyFnC₆₀, 6th contained 10⁻¹⁹ M HyFnC₆₀.

probability of discrimination between the 2 groups increased to 83–84%.

Figure 8 shows averaged kinetics for blood of patients in remission before treatment with Hypoxen® and after treatment, as well as the blood of patients in a state of acute attack, Figure 9 shows the kinetics of CL of blood of patients in the presence of 10^{-6} M of HyFnC₆₀ (Figure 9A), and 10^{-19} M of HyFnC₆₀ (Figure 9B). In these graphs we can see the difference between kinetics of CL of blood before and after treatment and in the state of acute attack, and the effects of HyFnC₆₀ upon CL parameters.

DISCUSSION

Recent estimations have shown that in adults >15% of oxygen consumed is turned into ROS under resting conditions and much more when their activity is high [8]. The process of one-electron oxygen reduction, in which energy of electronic excitation is generated, continuously occurs in human blood [9]. We have previously shown that LM-CL from undiluted whole blood of angina pectoris patients significantly exceeds that of blood of healthy donors, and its intensity diminishes in the course of successful treatment [10].

In this study it has been shown that Hypoxen® treatment significantly lowers the possibility of intense LM+ZM- and LC-induced ROS production in whole undiluted blood of COPD patients *in vitro*. In numerous parallel CL measurements it is shown that without Hypoxen® treatment and without addition of HyFnC₆₀ ROS production is comparatively more intense in whole blood. 10^{-6} M and 10^{-19} M concentrations of HyFnC₆₀ were used in the study because they had the most statistically significant impact on ROS production rate. Interestingly, 10^{-19} M HyFnC₆₀ alone added to blood of patients without Hypoxen® treatment remarkably lowered ROS production (by more than 50%, Figure 5). Hypoxen® treatment promoted even lower ROS production than in the latter case. Addition of HyFnC₆₀ to blood *in vitro* in a concentration of 10^{-6} M noticeably enhanced the effect of Hypoxen® and 10^{-19} M brought ROS production almost to the zero level. This data refers both to LM+ZM and LC-dependent ROS generation. The above data were obtained from patients with a chronic course of COPD. The same pattern of HyFnC₆₀ effect without Hypoxen® treatment was observed with blood of patients suffering an acute attack, except that 10^{-6} M concentration of HyFnC₆₀ had a larger effect on ROS production decrease than 10^{-19} M did. Comparing Figure 5 and Figure 6 it is clear that ROS generation rate is more than twice as intense in case of an acute attack than in a chronic case. HyFnC₆₀ are 12% more effective in case of acute attack.

Recently, peculiar antioxidant properties of HyFnC₆₀ were described [11]. Fullerenes are closed spherical polyhedrons entirely constructed of three coordinated carbon atoms. Fullerene C₆₀ is the most stable compound, with 12 pentagonal and 20 hexagonal rings [12,13].

Fullerenes are insoluble in water but they can be hydrated without their modification or use of detergents. This method allows us to produce a genuine solution of fullerenes in which each C₆₀ molecule is covered by a rather thick stable water shell that may consist of multiple water layers [14,15]. Fullerene molecules in HyFnC₆₀ preparations cannot directly

interact with any other molecules besides water of aqueous shells, and it is considered that their multiple biological effects including antioxidant effects are related to structured water that represents a unique biologically active agent [16].

Currently, much data has been accumulated on the potential use of fullerenes in medicine and biology [17]. Hydrated fullerenes (HyFnC₆₀) in low concentrations cause nonspecific antibacterial, antiviral and anti-inflammatory effects, and a neuroprotective effect in alcoholism [18]. Of particular interest are studies in which the effect of hydrated fullerenes on cancers was studied [17]. As shown, no toxicity was traced during the whole period of the experiments [19].

In the presence of HyFnC₆₀ the effect of Hypoxen® became clearer and the 3 groups of the examined patients appeared more discrete, according to DA. Thus, HyFnC₆₀ help better identify differences between groups of patients studied.

Numerous studies have confirmed the antioxidant activity of fullerenes. Antiradical properties of fullerenes were usually explained by direct scavaging of free radicals by the fullerene carbon cage. However, most recent studies show that numerous effects of HyFn upon a wide range of processes going on both *in vivo* and *in vitro* may be related both to their ability to make the aqueous environment in which these processes go on more ordered, and simultaneously to modulate redox reactions that continuously proceed in aqueous systems [20]. It had been shown previously that antioxidants can function in very low concentrations [21], supposedly via water structuring, which is consistent with the data of this study.

CONCLUSIONS

Hypoxen® treatment of COPD patients resulted in significant alternation of LM-ZM-dependent and LC-CL of whole blood of patients. This is consistent with the claimed strong antioxidant activity of Hypoxen®. HyFnC₆₀ added to blood *in vitro* enhanced the effect caused by Hypoxen®, which was given *in vivo*. Moreover, HyFnC₆₀ lowered standard deviation values of the data. Interestingly, not all the studied HyFnC₆₀ concentrations equally influenced the ROS production rate – the best effect upon DA between different groups of patients was caused by HyFnC₆₀ in concentrations of 10^{-6} M and 10^{-19} M.

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REFERENCES:

1. MacNee W, Tudor RM: 2 New paradigms in the pathogenesis of phronic obstructive pulmonary disease. *Proc Am Thorac Soc*, 2009; 6: 527–31
2. The Global Initiative for Chronic Obstructive Lung Disease (GOLD), 2007
3. Skyba P, Kluchova Z, Joppa P et al: Nutritional status in relation to respiratory impairment and systemic inflammation in patients with acute exacerbations of COPD. *Med Sci Monit*, 2009; 15(10): CR528–33

4. Pietras T, Szemraj J, Witusik A et al.: The sequence polymorphism of MnSOD gene in subjects with respiratory insufficiency in COPD. *Med Sci Monit*, 2010; 16(9): CR427–32
5. Cavalcante AGM, de Bruin PFC: The role of oxidative stress in COPD: current concepts and perspectives. *J Bras Pneumol*, 2009; 35: 1227–37
6. Bolevich SB: Bronchial asthma and free-radical processes. Moscow: Medicine, 2006 (in Russian)
7. Duke VA: Construction of psychodiagnostic tests: traditional mathematical models and algorithms. S.-P., 1994 (in Russian)
8. Voikov VL: Reactive oxygen species (ROS): pathogens or sources of vital energy? Part 1. ROS in normal and pathologic physiology of living systems. *J Alternat Complem Med*, 2006; 12: 111–18
9. Voikov VL, Asfaramov R, Bouravleva EV et al: Biophoton research in blood reveals its holistic properties. *Indian J Exp Biol*, 2003; 43: 473–82
10. Voikov VL, Novikov CN, Siuch NI: Alterations in Luminol-enhanced CL from nondiluted whole blood in the course of low-level laser therapy of angina pectoris patients. In: Cohn, G.E, Soper, S.A., eds. *Ultrasensitive Biochemical Diagnostics II*, SPIE Proc 1997, San Jose, CA, USA 2985: 286–94
11. Andrievsky GV, Kosevich MV, Vovk OM et al: On the production of an aqueous colloidal solution of fullerenes. *J Chem Soc Chem Commun*, 1995; 12: 1281–82
12. Kroto HW, Heath JR, O'Brien SC et al: C60: Buckminsterfullerene. *Nature*, 1985; 318: 162–63
13. Fowler PW, Manolopoulos DE: *An Atlas of Fullerenes*. Oxford: Clarendon Press, 1995
14. Andrievsky GV, Kosevich MV, Vovk OM et al: On the production of an aqueous colloidal solution of fullerenes. *J Chem Soc Chem Commun*, 1995; 12: 1281–82
15. Andrievsky GV, Kondakov IK, Vovk OM: Comparative analysis of two aqueous-colloidal solutions of C60 fullerene with help of FT-IR reflectance and UV-VIS spectroscopy. *Chem Phys Letters*, 2002; 364: 8–17
16. Andrievsky GV, Bruskov VI, Tykhomyrov AA, Gudkov SV: Peculiarities of the antioxidant and radioprotective effects of hydrated C60 fullerene nanostructures *in vitro* and *in vivo*. *Free Rad Biol Med*, 2009; 47: 786–93
17. Andrievsky GV, Burenin IS: On medicinal and preventive efficacy of small doses of hydrated C60 fullerenes at cancer pathologies. *Chemistry Preprint Archive*, 2002; 6: 53–68
18. Tykhomyrov AA, Nedzvetsky VS, Klochkov VK, Andrievsky GV: Nanostructures of hydrated C60 fullerene (C60HyFnC60) protect rat brain against alcohol impact and attenuate behavioral impairments of alcoholized animals. *Toxicology*, 2008; 1–8
19. Andrievsky GV, Klochkov VK, Derevyanchenko LI: Is C60 fullerene molecular toxic?! Fullerenes, Nanotubes and Carbon Nanostructures, 2005; 13: 363–76
20. Andrievsky G, Shakhnin A, Tronza A, Zhernosekov D, Tykhomyrov A: The acceleration of blood plasma clot lysis in the presence of hydrated C60 fullerene nanostructures in super-small concentration. *Fullerenes, Nanotubes and Carbon Nanostructures*, 2010; 18: 303–11
21. Pal'mina NP, Mal'tseva EL, Kurnakova NV, Burlakova EB: The effect of alpha-tocopherol over a wide range of concentrations (10(-2)-10(-17) M) on protein kinase C activity. Connection with proliferation and tumor growth. *Biochimika (Russian)*, 1994; 59: 193–200