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OXYGEN-TRANSPORT FUNCTION OF RATS' HEMOGLOBIN UNDER STREPTOZOTOCIN-INDUCED DIABETES MELLITUS AND ADMINISTRATION OF MEDICINAL MUSHROOMS *AGARICUS BRASILIENSIS* AND *GANODERMA LUCIDUM* CONDITIONS

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Structural changes and redistribution of hemoglobin types which are observed in hyperglycemia lead to disturbances of functional properties of this protein. As a result, impairment of hemoglobin oxygen-transport function promotes tissue hypoxia. This study shows that under streptozotocin-induced diabetes in rats observed the increase of carboxyhemoglobin content, the oxyhemoglobin dissociation curve shift to the left, and the decrease of P_{50}. These changes indicate the growth of hemoglobin affinity to oxygen and reduction of O_2 delivery to tissues. It should be noted that in diabetic animals increased level of glycosylated hemoglobin was observed. It has a higher affinity to oxygen than normal hemoglobin. Also the content of fetal hemoglobin increased that is a compensatory reaction of organism to the development of hypoxic condition. Administration of medicinal mushrooms *A. brasiliensis* and *G. lucidum* submerged cultivated mycelium powder to diabetic animals causes the returning of abovementioned indices to control values. Obtained results indicate the recovery of physical, chemical and functional properties of hemoglobin – main protein of red blood cells.

Keywords: streptozotocin, diabetes mellitus, hemoglobin, medicinal mushroom.

Besides hyperglycemia one of characteristic manifestation of diabetes is tissue hypoxia. It can develop as a result of oxygen transport system functioning violations and/or as a result of oxygen utilization increased rate under decreased supply of O_2 to tissues. Since body aerobic capacity determined mainly by the oxygen delivery therefore the disturbances of oxygen transport to tissues should be considered as a leading cause of hypoxia development in patients. Hypoxia in clinical cases is always a secondary phenomenon and disappears when its cause disappear (i.e. when the disease is eliminated).

The main component of oxygen transport system of blood is hemoglobin (Hb) – heterotetrameric protein with heme groups which stabilize the structure of protein, provide its correct folding and play crucial role in oxygen transport [6]. Thus, the change of iron atom valence (e.g. its oxidation to Fe^{3+}) leads to disturbances in hemoglobin oxygen transport function [23].

Oxyhemoglobin dissociation curve (ODC) is the main characteristic of hemoglobin ability to bind and release oxygen. ODC graphically shows the dependence saturation of Hb with oxygen from partial tension of O_2 in gas phase. Sigmoid shape of this curve is due to the conformational changes of hemoglobin during the first stage of oxygenation (transition from the so-called T-state (a tense, deoxygenated state with a low affinity to O_2) to R-state (a relaxed, oxygenated state with a high affinity to O_2)). Therefore the following oxygenation steps occur much easily as equilibrium constant of the next stages of Hb-oxygen interaction is higher than equilibrium constant of

the first step. This effect is caused by interaction between heme groups of Hb molecule and called cooperative effect. The main parameter of ODC is P_{50} – oxygen tension (pO_2) at which hemoglobin is 50% saturated. It characterizes the ability of hemoglobin to deliver oxygen to tissues [17]. High or low values of P_{50} indicate decreasing or increasing of hemoglobin affinity to oxygen, respectively. Changes in concentration of some metabolites (e.g. 2,3-diphosphoglycerate, CO_2, protons (change of pH)) can affect the P_{50} value [15]. Moreover, different types of hemoglobin have different affinity to oxygen that can affect the oxygen delivery to tissues, especially when there is redistribution of Hb types under condition of certain pathologies [17]. There is evidence which indicate that fetal hemoglobin (HbF, which is typical for early stages of ontogenesis) has higher affinity to oxygen which provides oxygen in sufficient quantity [1]. Meanwhile, glycosylated hemoglobin (HbA1c that is formed by irreversible non-enzymatic binding of glucose residues to beta-chains of hemoglobin) caused ODC shift to the left indicating the growth of Hb affinity to oxygen but, at the same time, indicate the lowering of tissues oxygenation [5, 16]. Therefore, it is believed that increased content of HbA1c caused by hyperglycemia is one of the factors of tissue hypoxia development [18].

The hemoglobin oxygenation level critically influences intracellular signaling pathways, action of hormones and/or vasoactive agents, ion transport, and deformability of red blood cells (RBC) [17]. These affect rheological properties of blood, which violations are one of the causes of development of diabetes mellitus secondary complication such as microangiopathies and macroangiopathies. They lead to development of tissue and organ hypoxia and damages observed in 1/3 –1/2 patients suffering from diabetes [3, 11].

Therefore, the aim of our study was to investigate the effect of *Agaricus brasiliensis* and *Ganoderma lucidum* submerged cultivated mycelium powder (SCMP) on oxygen transport function of rats' hemoglobin under the condition of streptozotocin-induced diabetes mellitus.

MATERIALS AND METHODS

The study was conducted on Wistar outbred white male rats weighing 150-180 g. The animals had free access to food and water in normal conditions of the vivarium (a 12-hour light and darkness cycle at 22°C ± 2°C and 55% ± 5% relative humidity). The experiments were performed in accordance with ethical code of Ukraine Ministry of Health.

The rats were divided into the following six groups: 1 – control (CON); 2 – control animals treated with A. brasiliensis powder (COA); 3 – control animals treated with G. lucidum powder (COG); 4 – streptozotocin-induced diabetic rats (STZ); 5 – streptozotocin-induced diabetic rats treated with A. brasiliensis powder (STA); 6 – streptozotocin-induced diabetic rats treated with G. lucidum powder (STG). Each group consisted of 6-8 rats.

Diabetes mellitus (DM) was induced by a single intraperitoneal injection of streptozotocin (Sigma, USA) in the dose of 50 mg/kg of body weight dissolved in 10 mM citrate buffer (pH=5.5). Blood glucose concentrations were measured in 72 hours and in 14 days after the induction of DM by the glucose oxidase method with the help of a reagent kit ("Felisit-Diagnostyka", Ukraine). The animals used in the experiment had glucose levels exceeding 14 mM. On day 15, after the diabetic induction, the rats began to receive a daily dose of 1 ml of SCMP of the mushrooms prepared in saline in the ratio corresponding to a dose of 1 g/kg of body weight. The preparation was administered perorally using feeding needles for 14 days. On day 15 of the powder administration, the rats were decapitated under light ether anesthesia. Blood samples were taken for the study with the addition of heparin (heparin:whole blood 50 U:1 ml).

Mushrooms' strains were obtained from the culture collection of Mycolivia Ltd. (Israel) and cultivated by method of submerged culturing [24].

The hemoglobin affinity to oxygen was determined spectrophotometrically using the oxygen saturation curve method [7, 22]. P_{50} values were calculated based on saturation curves.

Determination of hemoglobin ligand forms was performed in whole blood by absorption spectroscopy method [2].

The Student *t*-test was used for statistical analysis of data. $P<0.05$ was considered significant

RESULTS AND DISCUSSION

Assessing the effect of streptozotocin-induced diabetes mellitus on rats' erythron system the most important task was to determine the concentration of total hemoglobin. According to data published in our previous paper, we did not find any changes in total hemoglobin concentration in all experimental groups of rats [24].

As it was mentioned previously, glycosylated Hb has higher affinity to oxygen than adult hemoglobin and can affect tissue oxygenation. So, the next step was to determine its content in RBC of rats. The analysis showed significant HbA1c increase in diabetic rats. Administration of medicinal mushrooms SCMP to sick animals reduced this parameter to values of control group. These data was also published in our previous paper [24].

Modification of the globin part of hemoglobin (caused by different factors) leads to changes of its ligand form distribution that affects physicochemical properties, hemoglobin functional state and functionality of oxygen-transport system of the body. Therefore, analysis of hemoglobin ligand forms by absorption spectroscopy, a highly sensitive method, which was developed by the Department of Biochemistry of Ivan Franko Lviv National University, was performed.

Obtained results showed a significant increase by 12.7 % of carboxyhemoglobin (HbCO) content in RBC of diabetic rats compared with control animals (Table). In normal conditions, carbon monoxide (CO) is synthesized mainly by heme oxygenase, which catalyses heme catabolism to equimolar amounts of CO, iron, and biliverdin. CO has a high affinity to heme-containing proteins, especially to hemoglobin, and forms with it HbCO which decays 200 times slower than oxyhemoglobin [8]. The growth of carboxyhemoglobin content under DM is associated with the increase of endogenous pool of CO due to premature elimination and destruction of erythrocytes [4], and also with enhancement of heme oxygenase (HMOX) activity, in particular HMOX-1 – an inducible form of the enzyme which plays an adaptive role in stress [9, 10]. HMOX-1 can be quickly activated by changes of oxygen partial pressure, ROS, increased content of free heme [13]. The growth of HbCO content affects the affinity of hemoglobin to oxygen and the development of hypoxia. The proof of hypoxia development is the shift of ODC to the left (Figure, A) compared with the one in the control group and a decrease of P_{50} value (Figure, B).

Intracellular metabolism also can influence the oxygen-transport function of blood. There are reasons that indicate inhibition of 2,3-DPG production observed under hyperglycemia leads to the growth of hemoglobin affinity to oxygen that complicates its release in tissues [19, 21], and, thus, promotes hypoxia development. Enhancement of non-enzymatic glycosylation of hemoglobin blocks the interaction sites of 2,3-DPG that constrain allosteric regulation of oxygen-transport function of oxyhemoglobin. Thus, HbA1c promotes reduction of tissue oxygenation, but, on the other hand, formation of glycosylated hemoglobin can be an adaptive response of the body to an increased oxygen capacity of peripheral blood [14].

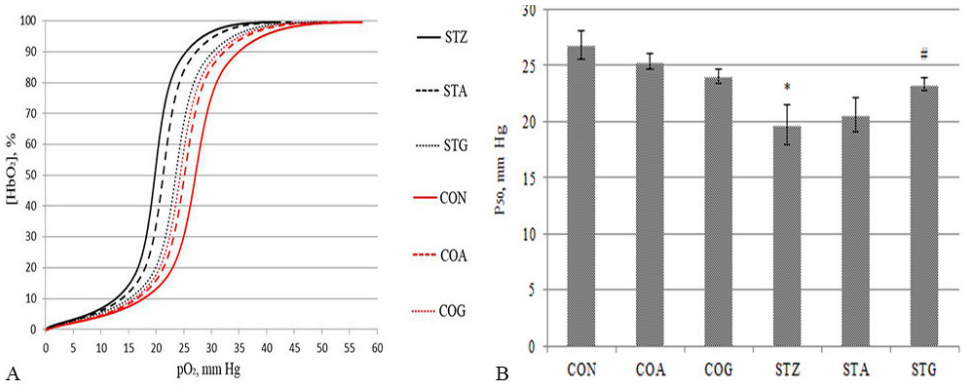
Administration of *A. brasiliensis* and *G. lucidum* SCMP to control animals led to reduction of HbCO content by 21.4 % and 26.6 %, respectively, compared with the CON group. Also was observed a decreased content of carboxyhemoglobin to control values in STA and STG groups (by 14.4 % and 12.8 % respectively) (Table).

Affinity to oxygen is an important property of hemoglobin that ensures its functionality. Oxyhemoglobin dissociation curve graphically displays the process of oxygen binding by hemoglobin. The character of ODC changes under different pathologies especially those that accompanied by hypoxia development. The main parameter of oxyhemoglobin dissociation curve is P_{50} value [20].

Content of specific ligand forms of hemoglobin in control (CON), streptozotocin-induced diabetic (STZ) and SCMP-treated rats

Groups	Hemoglobin ligand forms, %	
	HB (OXY-HB)	HbCO (carboxy-Hb)
CON	95.37 ± 0.29	4.63 ± 0.29
COA	95.08 ± 2.11	3.64 ± 0.66 *
COG	96.60 ± 0.20	3.40 ± 0.22 *
STZ	94.79 ± 0.34	5.22 ± 0.34 *
STA	95.53 ± 0.68	4.47 ± 0.68 #
STG	95.45 ± 0.37	4.55 ± 0.36 #

Notes: COA, control animals treated with *A. brasiliensis* powder; COG, control animals treated with *G. lucidum* powder; STA, streptozotocin-induced diabetic rats treated with *A. brasiliensis* powder; STG, streptozotocin-induced diabetic rats treated with *G. lucidum* powder. Values expressed as the means ± SEM (n = 6–8 per group). * $P<0.05$, all groups vs. CON group. # $P<0.05$, all groups vs. STZ group.



Typical oxyhemoglobin dissociation curves (**A**) and P_{50} values (**B**) in control (CON), streptozotocin-induced diabetic (STZ) and SCMP-treated rats. COA, control animals treated with *A. brasiliensis* powder; COG, control animals treated with *G. lucidum* powder; STA, streptozotocin-induced diabetic rats treated with *A. brasiliensis* powder; STG, streptozotocin-induced diabetic rats treated with *G. lucidum* powder. Values expressed as the means ± SEM (n = 6–8 per group). * $P<0.05$, all groups vs. CON group. # $P<0.05$, all groups vs. STZ group.

It was shown that ODC shifted to the left and P_{50} decreased by 26.4 % in SDM rats compared with control rats (Figure). Results indicate hypoxia development in diabetic animals [20]. In control groups of rats, which were administered SCMP of medicinal mushrooms, no significant changes in P_{50} values were found (Figure, B), whereas ODCs were slightly shifted to the left compared with control (Figure, A).

Oxyhemoglobin dissociation curves of SCMP-treated diabetic groups shifted to the right (towards control) (Figure, A) when compared with that one in non-treated diabetic. The P_{50} significantly increased by 18.0 % only in the STG group (Figure, B).

It should be noted that for the “fight” with hypoxia which develops in rats with streptozotocin-induced diabetes, the switch of hemoglobin beta-chains genes synthesis has occurred. As

a result, the content of HbF (which has higher affinity to oxygen and is capable to release O_2 at lower pO_2 than adult hemoglobin) grows [12]. Previously published data obtained by us show significant increase of fetal hemoglobin content in diabetic rats [24]. This leads to the ODC shift to the left and decreases of P_{50}. Intensification of fetal Hb synthesis is a compensatory response of the body to a decreased oxygenation and hypoxic state. Administration of medicinal mushrooms SCMP to control animals did not change the HbF content in RBC, while in diabetic animals its concentrations decreased to control values was observed [24].

Thus, the development of streptozotocin-induced DM in rats is accompanied by lowering of hemoglobin oxygen-transport function and leads to hypoxia development. Administration of medicinal mushrooms SCMP causes physicochemical and functional properties recovery of hemoglobin heterogenic system.

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КИСЕНЬ-ТРАНСПОРТНА ФУНКЦІЯ ГЕМОГЛОБІНУ ЩУРІВ ЗА УМОВ СТРЕПТОЗОТОЦИН-ІНДУКОВАНОГО ЦУКРОВОГО ДІАБЕТУ ТА НА ФОНІ ВВЕДЕННЯ МЕДИЧНИХ ГРИБІВ *AGARICUS BRASILIENSIS* І *GANODERMA LUCIDUM*

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Структурні зміни та перерозподіл типів гемоглобіну, що спостерігаються при гіперглікемії, призводять до порушення функціональних властивостей цього білка. Як наслідок порушується кисень-транспортна функція гемоглобіну, що

сприяє розвиткові тканинної гіпоксії. Проведені дослідження показують, що за умов експериментального цукрового діабету у щурів спостерігається збільшення вмісту карбоксигемоглобіну, крива дезоксигенації оксигемоглобіну зміщується вліво та знижується показник P_{50}. Це вказує на зростання спорідненості гемоглобіну до кисню та зниження його постачання у тканини. Крім того, у хворих тварин зростає рівень глікозильованого гемоглобіну, що має підвищену спорідненість до кисню, та збільшується вміст фетального гемоглобіну, що є компенсаторною реакцією організму на розвиток гіпоксичного стану. Введення порошкоподібного міцелію медичних грибів *A. brasiliensis* та *G. lucidum* хворим тваринам викликає повернення досліджуваних параметрів у межі контролю. Отримані результати свідчать про відновлення фізико-хімічних і функціональних властивостей основного білка еритроцитів – гемоглобіну.

Ключові слова: стрептозотоцин, цукровий діабет, гемоглобін, медичні гриби.

КИСЛОРОД-ТРАНСПОРТНАЯ ФУНКЦИЯ ГЕМОГЛОБИНА КРЫС В УСЛОВИЯХ СТРЕПТОЗОТОЦИН-ИНДУЦИРОВАННОГО САХАРНОГО ДИАБЕТА И НА ФОНЕ ВВЕДЕНИЯ МЕДИЦИНСКИХ ГРИБОВ *AGARICUS BRASILIENSIS* И *GANODERMA LUCIDUM*

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Структурные изменения и перераспределение типов гемоглобина, которые наблюдаются при гипергликемии, приводят к нарушению функциональных свойств этого белка. В результате нарушается кислород-транспортная функция гемоглобина, что способствует развитию тканевой гипоксии. Проведенные исследования показывают, что в условиях экспериментального сахарного диабета у крыс наблюдается увеличение содержания карбоксигемоглобина, кривая дезоксигенации оксигемоглобина смещается влево и снижается показатель P_{50}. Это указывает на рост сродства гемоглобина к кислороду и снижение его поставок в ткани. Кроме того, у больных животных растет уровень гликозилированного гемоглобина, который обладает повышенным сродством к кислороду, и увеличивается содержание фетального гемоглобина, что является компенсаторной реакцией организма на развитие гипоксического состояния. Введение порошкообразного мицелия медицинских грибов *A. brasiliensis* и *G. lucidum* больным животным вызывает возврат исследуемых параметров в пределы контроля. Полученные результаты свидетельствуют о восстановлении физико-химических и функциональных свойств основного белка эритроцитов - гемоглобина.

Ключевые слова: стрептозотоцин, сахарний диабет, гемоглобин, медицинские грибы.