GALLOCYANINE-BASED SCREENING METHOD FOR SUPEROXIDE DISMUTASE-LIKE AGENTS

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One of the most relevant areas in medical biophysics is screening of compounds capable to correct the development of oxidative/halogenative stress that accompanies many significant diseases. An important source of reactive oxygen species (ROS) and halogens (RHS), initiating the development of oxidative/halogenative stress, are neutrophils – the most abundant leukocytes in the blood which are constitute the first line of host defense against numerous infectious pathogens [1]. ROS have a crucial role in human physiological and pathophysiological processes. However, prolonged exposure to high ROS concentrations may lead to diseases (e.g., cardiovascular and neurodegenerative diseases), for which screening of selective antioxidant drags is required [2].

In the field of antioxidant therapeutics, ongoing researches are conducted to better understand the mechanism of action of known antioxidant agents and to design and test novel therapeutic agents [3]. So, problem of searching for specific and sensitive probes for ROS detection is being actively studied. Recently the fluorescence method of measuring the kinetics of superoxide ($^{\bullet}O_2^-$) production by monitoring of the gallocyanine – C.I.51030 (GC) dye bleaching, which is accompanied by an increase in the fluorescence intensity of the dye solution in cell suspensions has been proposed [4]. Using this method, the effect of well-known agents, such as superoxide dismutase (SOD), ceruloplasmin (CP), cysteine (Cys), N-acetylcysteine (NAC), taurine (Tau) and acetaminophen (APAP) on $^{\bullet}O_2^-$ neutrophil production has been tested.

Sodium citrate, phorbol 12-myristate 13-acetate (PMA), GC, SOD, CP, Tau, Cys, NAC, APAP were obtained from "Sigma", USA; dextran T70 – from "Roth", Germany; histopaque – from "Nycomed", Norway; others – from "Reachem", Russia and "Belmedpreparaty", Belarus. Neutrophils were isolated from venous blood of healthy donors as described elsewhere [5]. Cells were suspended in a phosphate buffered saline (PBS) containing 10 mM Na_2HPO_4/KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, 0.9 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose (pH 7.4) and stored at 4 ºC. Fluorescent characteristics of GC (5 μM in PBS) bleaching were registered on a spectrofluorimeter CM 2203 "Solar" (Minsk, Belarus), $\lambda_{ex.}$=360 nm, $\lambda_{em.}$=490 nm. Oxidation rate (v), defined as the slope of the initial linear portion of the fluorescence intensity curve, and reaction amplitude (h), defined as changes in fluorescence intensity of the solution compared to the background level at 7 min, were used to describe this process.

Data obtained in the study of SOD, CP, Tau, Cys, NAC, APAP effect on the fluorescent properties changes of GC in suspensions of 50 nM PMA-activated neutrophils (1×10^6 cells/ml) are given in the table 1.

Table 1. Anti-inflammatory effects of test substances (% of the PMA effect, *$p < 0.05$ compared to PMA effect).

	Control	SOD, 50 mg/l	CP, 150 mg/l	Tau, 3.2 mM	Cys, 3.3 mM	NAC, 500 μM	APAP, 100 nM
v, %	100	10±7*	11±10*	105±15	19±18*	60±3*	101±13
h, %	100	15±10*	40±25	104±17	44±32	62±9*	96±22

The results presented in table 1 indicate that SOD and CP, which has superoxide dismutase-like activity, inhibit the production of $^{\bullet}O_2^-$ compared to the control (PMA only). Tau, a hypochlorite interceptor, did not affect the changes in the fluorescence parameters of the solution. Cys and NAC containing sulfhydryl groups are quite good scavengers for ROS, as can be seen from table 1. It is known that APAP has antipyretic (cyclooxygenase-3 and myeloperoxidase inhibitor), but not anti-inflammatory effect [6], which is also confirmed by our research (see table 1). Given together, the data obtained in the study has been showed that GC is a promising dye for screening SOD-like activity of drugs and for better understanding the mechanism of their action. This study was supported by Russian Foundation for Basic Research (18-515-00004) and Belarusian Republican Foundation for Fundamental Research (B18R-058).

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