ACETAMINOPHEN AS A REGULATOR OF NEUTROPHILS' ROS AND RHS PRODUCTION

Veronika Reut[1], Daria Grigorieva[1]

[1] Department of Physics, Belarusian State University, Belarus
ReutVE@bsu.by

Acetaminophen (APAP) is a frequently prescribed over-the-counter drug to reduce fever and pain in the event of inflammatory process. As neutrophils are relevant cells in inflammatory processes, the putative interaction of APAP with these cells or with reactive oxygen (ROS) and halogens (RHS) species, produced by them, is of a great importance [1]. As oxidative stress is due to primary antioxidant deficiency or to excess production of ROS, attention should aim to scavenge ROS or to inhibit their production [2]. The present study was undertaken to evaluate the effect of APAP in human neutrophils' superoxide ($\bullet O2^-$) and hypochlorous acid (HOCl) production.

In previous studies it was shown that gallocyanine (GC) and celestine blue B (CB) dyes are sensitive sensors of "turn-on" type to $\bullet O2^-$ and HOCl-derivatives production respectively [3].

Sodium citrate, phorbol 12-myristate 13-acetate (PMA), N-formyl-met-leu-phe (fMLP), GC, CB, 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 [4]. Cells were suspended in a phosphate buffered saline (PBS) containing 10 mM Na2HPO4/KH2PO4, 137 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl2, 0.5 mM MgCl2, 5 mM D-glucose (pH 7.4) and stored at 4 ºC. Changes in fluorescent characteristics of GC (5 μM in PBS, λex.=360 nm, λem.=490 nm), CB (20 μM in 20 % water glycerin solution, λex.=460 nm, λem.=590 nm) were registered on a spectrofluorimeter CM 2203 "Solar" (Minsk, Belarus). 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 (for GC) or 15 min (for CB), were used to describe this process.

Data obtained in the complex study of 100 μM APAP effect on chemosensors fluorescent properties changes in suspensions of 50 nM PMA or 0.5 μM fMLP-activated neutrophils (1×10^6 cells/ml) are given in the table 1.

Table 1. Effect of 100 μM APAP on chemosensors fluorescent properties (% of stimulant effect, *$p < 0.05$ compared to stimulant effect).

stimulant	parametr	GC	CB	stimulant	parametr	GC	CB
fMLP	*v*, %	153±38	-	**PMA**	*v*, %	125±28	226±30*
	h, %	179±39*	-		*h*, %	129±11*	201±12*

The results presented in table 1 indicate that APAP at in vivo relevant therapeutic concentration enhance both $\bullet O2^-$ and HOCl production compared to the control (stimulant only) its well-known role of H2O2 scavenger. It is notably that in cell-free system APAP directly interacts with HOCl to produce chlorinated adducts as has been shown by increase of CB fluorescence intensity (data not shown). Based on our results, it follows that using CB method to test the activity of neutrophils in the presence of pharmacological preparations, it is necessary to take into account their possibility of modification with the formation of halogenated products that will directly interact with this dye. According to results undertaken, it is necessary to limit the concentration of the drug in plasma to those where the effect of the peroxide interceptor is strong, but there is no such accumulation of halogenated products (corresponding a single oral dose less than 1 gram).

This study was supported by Russian Foundation for Basic Research (19-54-04004) and Belarusian Republican Foundation for Fundamental Research (B18R-058).

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