ROS PRODUCTION AND DNA STATUS IN HUMAN LYMPHOCYTES FOLLOWING ALUMINUM CHLORIDE EXPOSURE

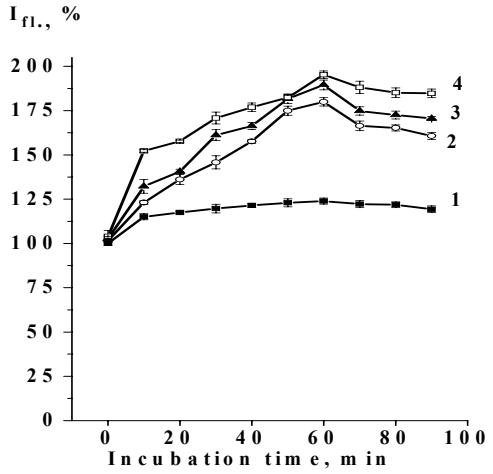
Skarabahatava A.S.[1], Lukyanenko L.M.[1], Slobozhanina E.I.[1], Falcioni G.[2], Falcioni M.L.[2]

[1]Institute of Biophysics and Cell Engineering of the National Academy of Sciences of Belarus, sas.alesya@gmail.com
[2]School of Pharmacy and Health Products, University of Camerino, Italy

Aluminum has no known essential role within the body and no useful biological function for it has been discovered. However, its accumulation may affect a variety of biological functions. Diseases have been associated to high levels of Al. In spite of numerous efforts, the mechanisms of Al toxicity has to be better delineated.

In this ambit our interest has been devoted to human lymphocytes and in particular we have detected the production of reactive oxygen species (ROS) and the DNA status following the *in vitro* Al exposure of these blood cells.

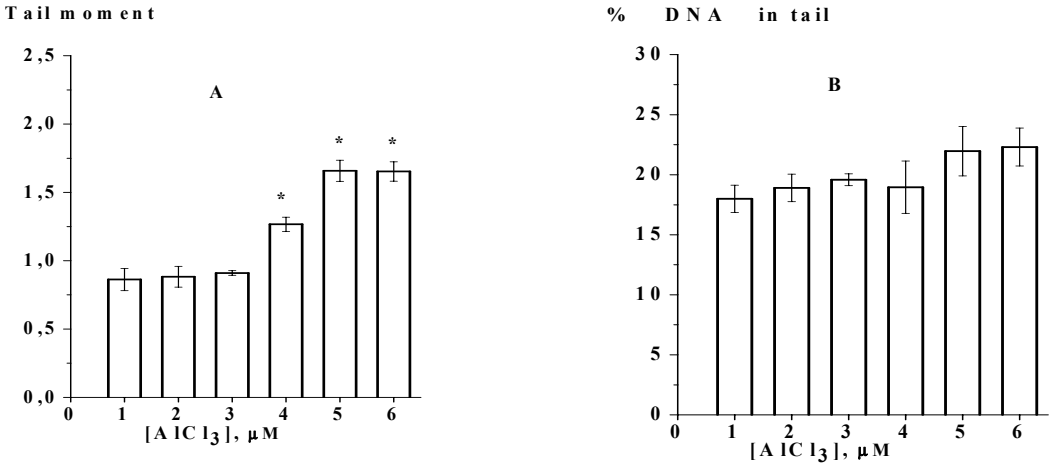
Detection of the overall intracellular generation of reactive oxygen species (ROS) was done by carboxy-H_2-DCFDA. This compound added to the cells diffuses across the cell membrane and is hydrolyzed by intracellular esterases to carboxy-H_2-DCF which, upon oxidation, is transformed into highly fluorescent DCF. It is evident from Fig.1 that the amount of ROS, directly proportional to the fluorescent intensity, is increased following aluminum exposure. Under our experimental conditions, $AlCl_3$ induces a dose-dependent increasing effect for at least 1h. After this period the decrease of fluorescence observed could be due to photobleaching.



1 – control; 2 - 75 μM $AlCl_3$; 3 – 100 μM $AlCl_3$; 4 - 150 μM $AlCl_3$.

Figure 1 – Aluminum chloride influence on fluorescent intensity changes of CM-H_2DCFDA in lymphocytes (λ_{ex} =505 nm; λ_{em} =525 nm)

In the present study, we also explored the effect of $AlCl_3$ on the status of lymphocyte DNA using the “comet assay“ or “single-cell gel electrophoresis”.



1 — control; 2 – 10 μM $AlCl_3$; 3 – 25 μM $AlCl_3$; 4 – 50 μM $AlCl_3$; 5 – 75 μM $AlCl_3$; 6 – 100 μM $AlCl_3$.

Figure 2 – Tail moment (A) and % DNA in tail comet parameter (mean ± s.e.m.) measured on human lymphocytes suspension incubated in the presence and absence of different concentration aluminum chloride (* - $p<0.05$)

This is a popular tool for the measurement of DNA damage in individual cells. The extent of DNA damage was quantified by measuring the displacement of the genetic material between the cell nucleus (comet “head”) and the resulting “tail”. The parameters used (Fig.2) as an index of DNA damage are % tail DNA and tail moment (TM); the latter is an index of DNA damage which considers both the tail length and the fraction of DNA in the comet tail.

The comet assay was performed on human lymphocytes exposed for 3 hours at different amount of $AlCl_3$ (10-100 μM). The presence of $AlCl_3$ influenced (Fig.2) in a hetereogeneous manner the two comet parameters. A significant increase of TM compared to the control (absence of aluminum) was observed only when the salt concentration was higher than 50 μM. The % tail DNA instead resulted unchanged significantly for all the aluminum concentration used. This result is not indicating the presence of DNA damage following aluminum exposure given that the measure of % tail DNA is considered the most significative for evaluating DNA damage.

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