Fluoranthene induces changes leading to programmed cell death

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Group of polycyclic aromatic hydrocarbons (PAHs) belongs to the most significant pollutants of living environment with ability of deposition in living organisms. Their effect is well known on animals and people, but effect of these compounds on plants, especially on cellular and sub-cellular levels, is still almost unknown. Due to this fact, we were focused on influence of fluoranthene on plant cell model – cell suspension culture of Nicotiana tabacum cv. BY-2. Significant phytotoxicity and ability of fluoranthene to be accumulated in plant cells were demonstrated.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed organic pollutants, which are able to enter soil as well as aquatic systems with subsequent deposition in living organisms, especially thanks to their lipophilicity[1-5]. They are intensively studied due to their carcinogenic and mutagenic effects on animal and human cell lines. They demonstrate ability to induce oxidative stress as well as DNA damage, especially after activation by UV radiation. Oxidation stress is connected with generation of reactive oxygen species (ROS), which may serve as signals of initiation of processes leading to programmed cell death[6]. One of the recent studies demonstrates fact than activation of some PAHs (e. g. benz[a]anthracene, fluoranthene or pyrene) by UV radiation is not necessary to damage DNA[7]. Effect of PAHs on plants is still predominantly unknown, except of studies of some PAHs on model plant – *Arabidopsis thaliana*, where induction of oxidative stress was demonstrated[8]. Fluoranthene (FLT), member of PAHs group, is used as model for investigation of PAHs toxicity, especially because of its reduced toxicity in comparison with other PAHs. To better understand the biochemical and cytological responses to FLT, tobacco BY-2 cells as the most suitable plant cell model were treated by fluoranthene. Changes in cell structure and viability as well as nuclear architecture were monitored. In addition, ability of FLT to generate reactive oxygen species was also investigated.

2 Methods

Nicotina tabacum L. cv. BrightYellow-2 suspension-cultured cells (BY-2) were grown in liquid cultivation medium according to Murashige and Skoog, modified by Nagata[9] with constant shaking (Kuhner Shaker LT-W, Adolf Kuhner AG, Switzerland, 130 rpm) at 27°C in the dark in 250 ml Erlenmeyer flasks. Cells in exponential growth phase were exposed to fluoranthene (Sigma-Aldrich, USA, 1 $mg.ml^{-1}$stock solution in dimethyl sulfoxide) in concentrations from 0 to 1000 μM, namely 0, 25, 50, 100, 250, 500 and 1000 μM, samples were taken at 12, 24, 48, 72, 96 and 120 hours after FLT application. As second control, dimethyl sulfoxide (0.5 %, v/v) was used. Cell viability was detected using fluorescein diacetate (FDA, 2.4 $\mu mol.l^{-1}$) and propidium iodide (PI, 2.4 $\mu mol.l^{-1}$); this technique is based on esterase activity of all living cells, which are able to metabolize FDA to fluorescein (alive

cells), contrariwise, PI penetrates only through damaged biomembranes and subsequently intercalates DNA (death cells). Percentage of viable cells was expressed as percentage of cells with esterase activity related to all cells. Nuclear morphology including chromatin condensation and presence of apoptic-like bodies (fragmented DNA) was determined using Hoechst 33258 (cells were fixed by PEM buffer, pH = 6.9, containing 4 % formaldehyde (v/v)), generation of reactive oxygen species was visualized using dihydroethidium (all chemicals Sigma-Aldrich, USA). All observations were carried out using fluorescent microscope (Olympus AX70) equipped with broad-spectrum UV excitation. All experiments were carried out in triplicates; observed changes were expressed as a percentage of total cells.

3 Results and discussion

Treatment of BY-2 cells by fluoranthene led to significant changes in cell structure; changes in nuclear architecture were also determined. On the other hand, generation of reactive oxygen species was only minimal.

Application of fluoranthene induced important changes, which were well evident using double FDA and PI staining. These changes included significant changes in fluorescence, especially fluorescence of biomembranes, in higher FLT concentrations (250 μM and higher). Application of FLT led to visualisation of structures, which are normally indistinct – mitochondria (not shown). These changes could be induced by incorporation of FLT into biomembranes due to its lipophilicity, eventually by metabolic activation of fluoranthene itself. In addition, fluorescence of fluorescein originated by esterase activity was suppressed; contrariwise, in these cases, cells remained alive without staining by PI. As obtained results demonstrate, BY-2 cells stained by PI and FDA can be divided into three categories: i. BY-2 cells with esterase activity and without disruption of permeability of biomembranes (positive FDA, negative PI staining), ii. BY-2 cells without evident esterase activity and without disruption of permeability of biomembranes (negative FDA, negative PI staining) – "transient cells", and iii. BY-2 cells without esterase activity and with disruption of permeability of biomembranes (negative FDA, positive PI staining) (Fig. 1). This fact probably means that FLT is able to inhibit esterase activity, or cover fluorescence of fluorescein due to its accumulation in BY-2 cells.

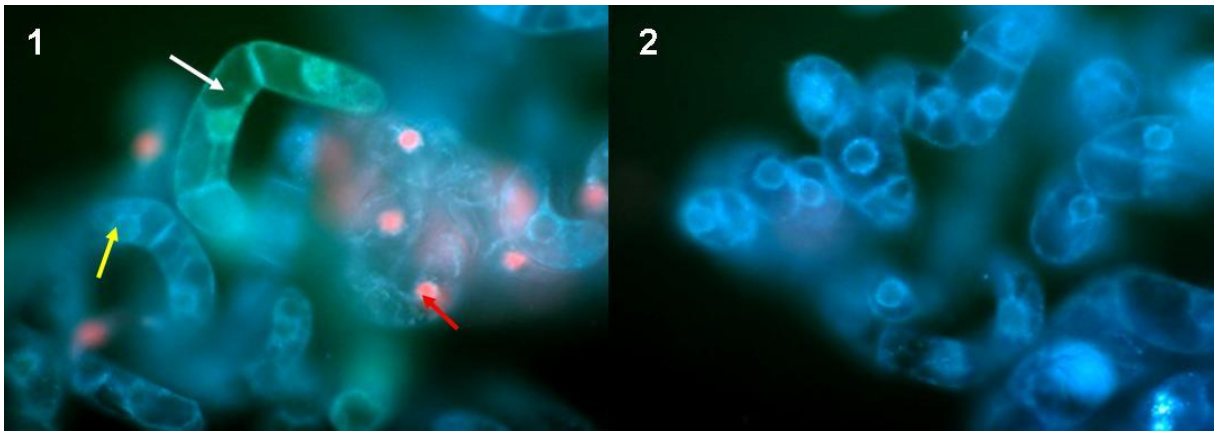

Fig. 1. Comparison of esterase activity and permeability of biomembranes of BY-2 cells treated by fluoranthene in concentration 1000 mM 72 h after application. Three types of BY-2 cells are distinguishable – BY-2 cells with esterase activity (white arrow), BY-2 cells without esterase activity and disruption of biomembranes permeability (yellow arrow), and BY-2 cells with disruption of permeability of biomembranes (red arrow) (1). Detail of BY-2 cells without disruption of permeability of biomembranes and esterase activity; apoptic-like bodies – DNA fragmentation – are well evident (white arrow) (2).

Viability of tobacco BY-2 declined depending on FLT concentration; the highest FLT concentration led to viability decline to approximately 50 % after 12 hours of treatment and

to 45 % after 120 hours of treatment (Fig. 2). Rapid viability decline in highest FLT concentration indicates ability of FLT to directly interfere with cell functions, which are crucial, e. g. permeability of biomembranes, or cell energetic metabolism (mitochondria).

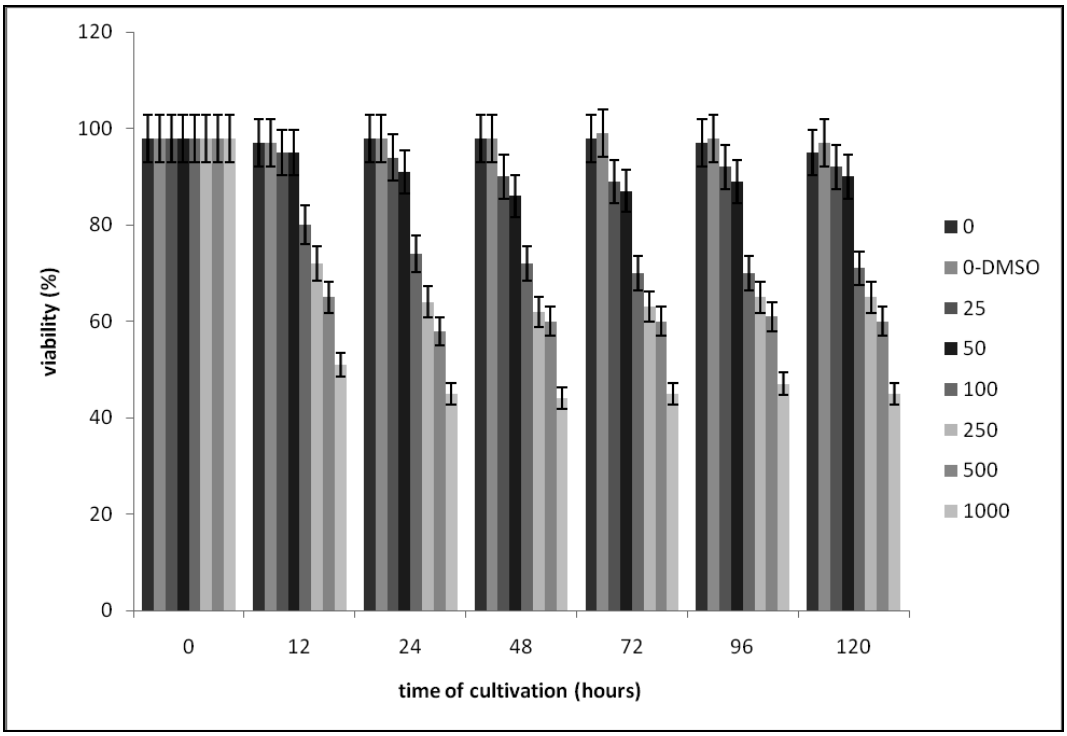

Fig. 2. Viability of tobacco BY-2 cells during treatment (0-120 hours) by different concentrations (0, 25, 50, 100, 250, 500 and 1000 μM) of fluoranthene. 0-DMSO represents BY-2 cells treated by dimethyl sulfoxide in concentration 0.5 % (v/v).

Due to occurrence of transient BY-2 cells, in next step, we were focused on determination of FLT accumulation, which can be responsible for changes in autofluorescence of BY-2 cells (Fig. 3). Obtained results indicate accumulation of FLT in cellular compartments, especially around nuclei, it means, FLT is accumulated in cells, probably in biomembranes of Golgi and endoplasmic reticulum.

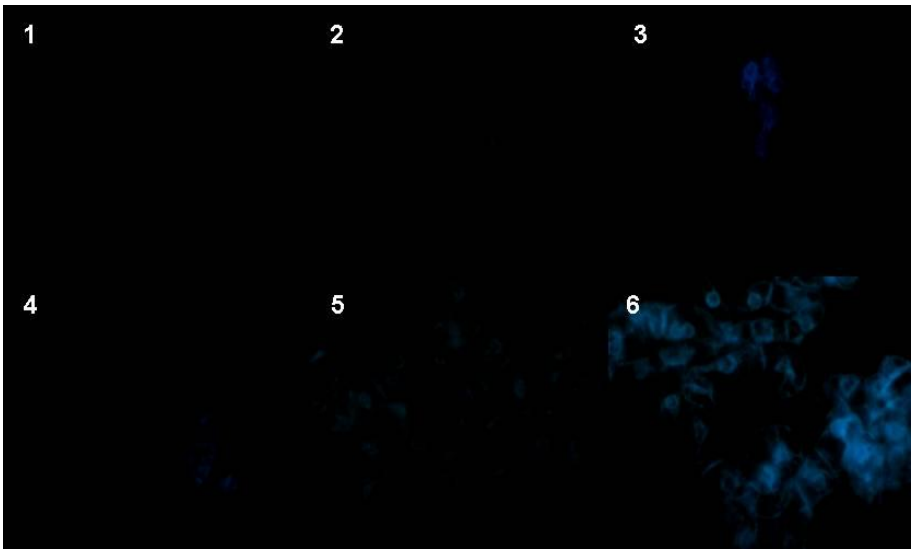

Fig. 3. Autofluorescence of BY-2 cells exposed to fluoranthene in concent-rations 0 (1), 50 (2), 100 (3), 250 (4), 500 (5) and 1000 (6) mM 72 h after application. Changes in fluorescence are cau-sed by accumulation of FLT in biomembranes of cell organelles.

FLT induces also significant nuclear changes, which include chromatin condensation, especially around nucleoli, and presence of apoptic-like bodies. Apoptic-like bodies indicate fact that FLT is able to induce programmed cell death. These changes were more evident in lower FLT concentrations. Programmed cell death is usually connected with reactive oxygen species, but may be connected with DNA damage and mitochondrial dysfunction. These explanations of FLT effect on BY-2 cells correlate with literature data, which were demonstrated on animal and human cell models[6,7].

4 Conclusion

FLT represents important polutant of living environment, which is able to enter food chain. Our experiments on BY-2 cells demonstrated its ability to be accumulated in plant cells, especially in biomembranes due to their amphipathic character (changes of fluorescence) as well as ability to induce processes leading to programmed cell death, which are manifested as chromatin condensation and DNA fragmentation. Mechanisms of these processes must be further studied.

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