Cell signals as markers of cytotoxicity of new complexes of naphthoquinones

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Naphthoquinones as group of secondary metabolites of plants demonstrate very interesting pharmacological properties, which are based on different mechanisms, especially on ability of interaction with DNA and generation of reactive oxygen species. Complexes of naphthoquinones with heavy metal can enhance these properties, especially cytotoxic. Due to this fact, we were focused on characterization of complex of naphthoquinone lawsone with copper(II) ions. This newly synthesized complex demonstrated significant cytotoxic, respectively phytotoxic properties.

1 Introduction

Naphthoquinones are less common secondary metabolites of some fungi, plants, but also bacteria, usually based on skeleton of 1,4-naphthoquinone. The most important naphthoquinones-containing plant families are *Bignoniaceae*, *Droseraceae*, *Ebenaceae*, *Juglandaceae*, and *Plumbaginaceae*[1,2]. Naphthoquinones have many physiological roles – ubiquinone, plastoquinone and K vitamins are functional constituents of biochemical systems. They are very interesting compounds with wide range of biological actions, including antibiotic, antiviral, antifungal, anti-inflammatory, antiproliferative and cytotoxic effects[1,3]. Cytotoxicity is one of the most important properties of naphthoquinones, which is based on generation of reactive oxygen species (ROS), disruption of mitochondrial functions, inhibition of thymidine incorporation into DNA as well as DNA intercalation[4,5]. Recently, it was determined that naphthoquinone plumbagin is able to inhibit invasion and migration of malignant cells, especially by inhibition of metalloproteinase-2 and urokinase-plasminogen activator[6,7]. Complexes of naphthoquinones with heavy metal ions may represent very interesting possibility of modification of their biological properties. For this purpose, new complexes of lawsone (2-hydroxy-1,4-naphthoquinone) with copper (Cu(II)) were synthesized and subsequently characterized using cell model of suspension cell culture of tobacco BY-2, which is compared to HeLA cells[8]. Submitted work is focused on characterization of the chemically simplest complex - $Cu(Law)_2(H_2O)_2.1/2H_2O$ – using plant cell model of tobacco BY-2 cells.

2 Methods

Nicotina tabacum L. cv. BrightYellow-2 suspension-cultured cells (BY-2) were grown in Murashige and Skoog liquid medium, modified by Nagata[8] 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 $Cu(Law)_2(H_2O)_2.1/2H_2O$ ($C_{20}H_{15}CuO_{8.5}$, M = 454.89, 1 $mg.ml^{-1}$stock solution in dimethyl sulfoxide), Cu(II) ions (as acetate, $1mg.ml^{-1}$stock solution in distilled water), and lawsone (1 $mg.ml^{-1}$stock solution in dimethyl sulfoxide) in concentrations from 0 to 1000 μM. As second

control, dimethyl sulfoxide (0.5 %, v/v) was used. Microscopical observations in brief: cell viability was measured using fluorescein diacetate (FDA) and propidium iodide (PI), nuclear morphology was determined using Hoechst 33258, determination 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

Application of $Cu(Law)_2(H_2O)_2.1/2H_2O$ as well as Cu(II) ions and lawsone in concentration range led to significant changes in BY-2 cells morphology and viability as well as changes of nuclear architecture, mitotic disorders and manifestation of symptoms of programmed cell death; in addition, generation of reactive oxygen species was also determined. Each control BY-2 cell demonstrated typical structure: presence of several vacuoles, well evident nucleus and several cytoplasmic strands separating individual vacuoles. In the case of applied complex (but not lawsone and Cu(II) ions), significant structural changes were well evident. These changes included indistinct separation of organelles and diffuse staining of cell by degradation product of FDA – fluorescein. These changes indicate ability of complex to damage permeability of biomembranes (Fig. 1).

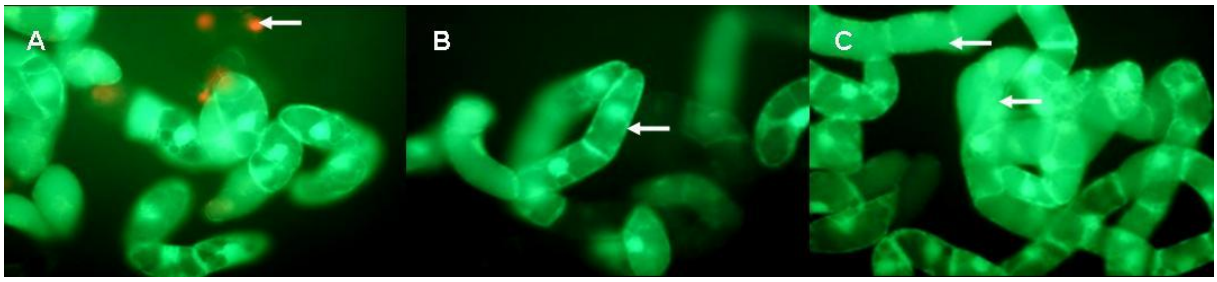

Fig. 1. Morphological changes of BY-2 cells stained by PI and FDA after treatment by prepared complex. Control BY-2 cells with a view to death cells (A, arrow), and BY-2 cells treated by complex in concentrations 100 mM (B) and 500 mM (C) 24 h after exposition. Typical changes are marked by arrows. Changes in fluorescence – diffuse staining of cells – are well evident. These changes show evidence of damage of permeability of biomembranes with maintenance of cell viability.

In addition, generation of reactive oxygen species was detected. These changes correlated with applied concentration of complex, in the case of lawsone and Cu(II) ions were not so evident.

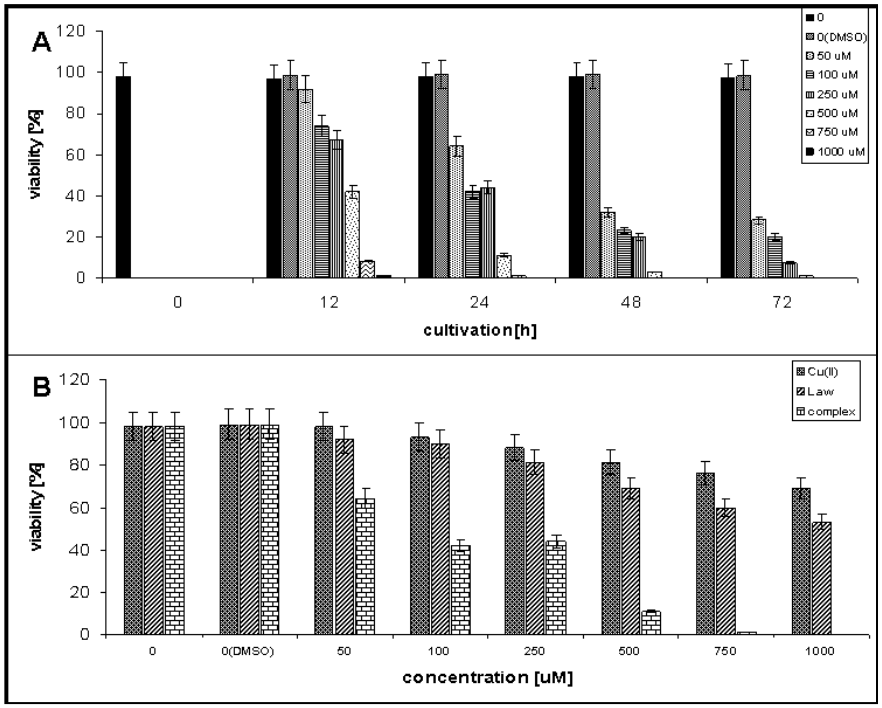

Fig. 2. Cytotoxic properties of complex, lawsone (Law) and Cu(II) ions. A. Cytotoxicity of complex in concentrations from 0 to 1000 μM. B. Comparison of cytotoxicity of cu(II) ions, lawsone (Law) and complex 24 hours after treatment.

All above mentioned changes led to decrease of cell viability, which was most significant in the case of complex in concentrations 500 μM and 1000 μM after 12 hours of treatment (Fig. 2). As it was determined previously, naphthoquinones are able to generated reactive oxygen species; this ability was determined also in the case of heavy metal ions[7]. The most important is hydrogen peroxide, which serves as signal inducing programmed cell death[9]. Application of complex led to typical cellular changes (cytoplasm shrinkage) as well as chromatin condensation and formation of apoptic-like bodies, which are manifestation of DNA fragmentation (Fig. 3).

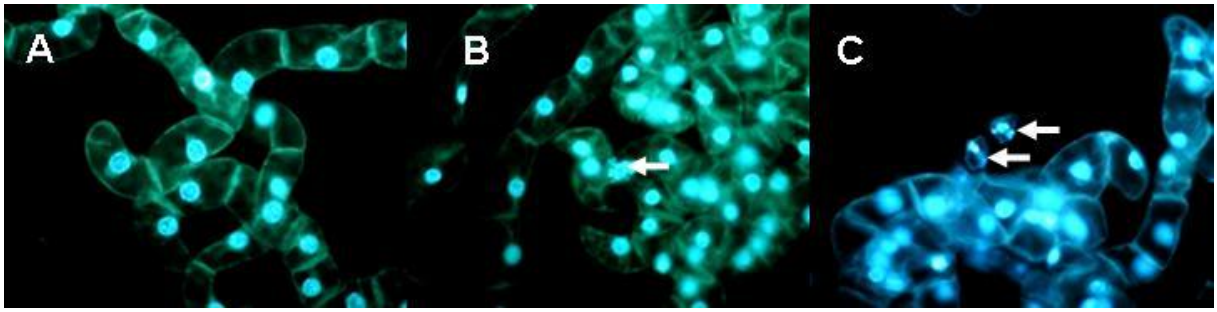

Fig. 3: Changes in nuclear architecture of BY-2 cells treated by complex, copper(II) ions and lawsone. Changes of nuclei 24 h after complex application are shown in A-C. A – control BY-2 cells without marks of DNA fragmentation, B – BY-2 cells treated by complex in concentration 100 mM and C – BY-2 cells treated by complex in concentration 250 mM. Arrows indicate presence of apoptic-like bodies.

These changes were well evident also in the case of Cu(II) ions and lawsone, but in higher concentrations in comparison with complex (Fig. 4).

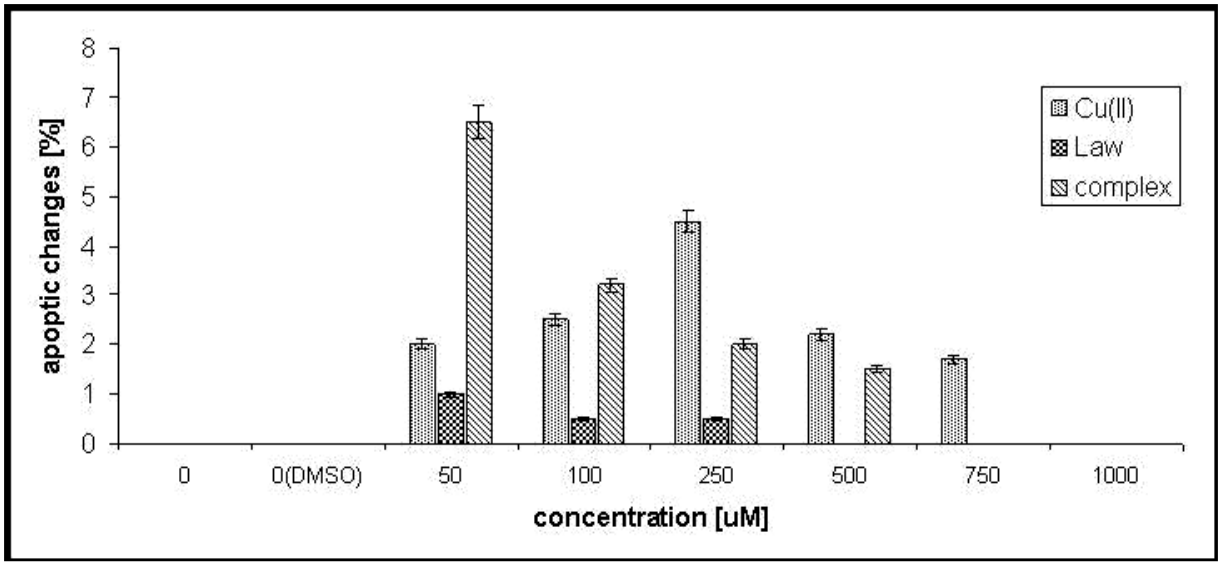

Fig. 3. Apoptic changes 24 hours after application of Cu(II), lawsone (Law), and complex in concentration range. All changes are expressed as percentage of occurrence of apoptic-like bodies in relation to all nuclei.

4 Conclusion

Tested complex demonstrated significantly higher cytotoxicity in comparison with lawsone and Cu(II) ions. Lower concetrations of complex led to manifestation of programmed cell death, contrariwise, its higher concentrations led to rapid cell death. In accordance with obtained results, we can state that complex in lower concentrations is able to generate signals leading to programmed cell death (ROS), at the other side in higher concentrations causes damage of biomembranes and probably interferes with cell metabolism, which leads to rapid cell death.

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