Journal of Trace Elements in Medicine and Biology 48 (2018) 141–148

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Biochemistry

Seleno-L-methionine and L-ascorbic acid differentiate the biological activity of doxorubicin and its metal complexes as a new anticancer drugs candidate

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ARTICLE INFO

Keywords:
Cancer
Doxorubicin
Metal complexes
Seleno-L-methionine
Vitamin C
Cytotoxicity
Genotoxicity

ABSTRACT

The most important problems of anti-cancer therapy include the toxicity of the drugs applied to healthy cells and the multi-drug cells resistance to chemotherapeutics. One of the most commonly used anticancer drugs is doxorubicin (DOX) used to treat certain leukemias and non-Hodgkin's lymphomas, as well as bladder, breast, stomach, lung, ovarian, thyroid, multiple myeloma and other cancers. Preliminary studies showed that metal complex with DOX improve its cytostatic activity with changes in their molecular structure and distribution of electrons, resulting in a substantial change of its biological activity (including antitumor activity). Thus, there is a chance to receiving derivatives of DOX with low toxicity for the healthy body cells, thus increasing its therapeutic selectivity. In the present study we examined the influence of Mn, Mg, Fe, Co and Ni, seleno-L-methionine and vitamin C on biological activity of DOX in prokaryotic model - *Escherichia coli* RFM443, with plasmid transcriptional fusion of *recA* promoter and *luxCDABE* as a reporter gene. Cytotoxic potency of tested chemicals was calculated on the basis of the bacteria culture growth inhibition (GI%) values. Genotoxic properties were calculated on the basis of the fold increase (FI) of relative luminescence units (RLU) values compared to control. Obtained results showed that doxorubicin metal complexes particularly with Ni, Co and Fe increased the cyto- and genotoxic activities of DOX. Bacteria culture supplemented with SeMet and vitamin C differentiate the DOX and its metal complexes toxicity. It seems, that DOX-Ni, DOX-Fe and DOX-Co complexes could be potent cytostatic drug candidates. Moreover, we noticed different sensitivity of *recA::luxCDABE* for 3 h and 24 h cultures of bacteria strain. It suggests, that the potency of genetic construct reactivity- *recA::luxCDABE* in *E. coli* depends on the growth-phase of bacterial culture.

1. Introduction

Doxorubicin (DOX) is an anthracyline natural drug extracted in 1970's from *Streptomyces peucetius var. caesius*. DOX is one of the most effective anti-tumor agent with antineoplastic effect, and is widely used as a first chemotherapeutic drug treatment in a variety of cancers (breast, lung and ovary), sarcomas, leukemia, and hematological diseases such as and Hodgkin's disease. Doxorubicin is a DNA intercalating agent that inhibits topoisomerase II activity and induces DNA breaks in cancer cells resulting in DNA damage and cell death. DOX is utilized for many years in cancer therapy. Unfortunately, treatment by this drug has been limited substantially, due to the significant toxicity that can occur during, but also some years after treatment. Various mechanisms have been ascribed to the DOX long-term induced toxicity. The main effect of DOX on a molecular level is directly connected with its ability to generate large amounts of reactive oxygen species (ROS). Moreover, DOX inhibits the ROS neutralizing enzymes, which are normally present in the tissue. Membrane lipid peroxidation and oxidative damage to other cellular components is the major factor in DOX toxicity. Additionally, DOX cannot effectively discriminate healthy cells from malignant ones, and it rapidly and non-selectively accumulates in healthy tissues. Once initiated, peroxidation continues autocatalytically, and has a progressive course that results in structural and functional changes both in treated and healthy tissues, resulting in severe clinical toxicities. DOX use in chemotherapy has been limited, largely due to its diverse toxicities, including cardiac, renal, pulmonary, hematological and testicular toxicity [1–4].

The most important problems in anti-cancer therapy include the toxicity of the drugs applied to healthy cells and the multi-drug resistance to chemotherapeutics. For these reasons, it is desirable to

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https://doi.org/10.1016/j.jtemb.2018.03.021
Received 15 November 2017; Received in revised form 16 March 2018; Accepted 23 March 2018

develop a new drugs modalities that can enhance anthracycline therapeutic efficacy and can decrease DOX cytotoxicity forward healthy cells [1–6].

Our current investigations conducted in collaboration with Medical University of Bialystok and MD Anderson Cancer Center (Houston, USA) are a part of "Study on improving the selectivity and activity of selected drugs and natural compounds with anticancer properties due to metal complexation". Preliminary results showed, that complex of ligand (with proven cytostatic activity) with metal, results in the change of its molecular structure and distribution of electron charge, resulting at the end in a change of its biological activity (including antitumor activity). We have chosen natural phenolic compounds found in plants, such as rosmarinic, caffeic, cichoric, elagic, chlorogenic acids, but also one of the most effective antineoplastic agents - doxorubicin. Our preliminary study indicated that, there is a chance to receive derivatives with low toxicity for the healthy body cells and increased therapeutic selectivity [4–6].

Metals play important role in medical biochemistry and they are a source for metal-based drugs with promising pharmacological application for treatment of several human diseases like carcinomas, lymphomas, infection, anti-inflammatory, diabetes, and neurological disorders. Up to now, only platinum in cisplatin is used, but antitumorous activity has been documented for some other metals: gallium, germanium, tin, bismuth, titanium, ruthenium, rhodium, iridium, iron, molybdenum, cobalt, copper, nickiel, gold. Transition metals exhibiting different oxidation state can interact with a number of negatively charged molecules. Complexes of transition metals like iron show remarkable anti-proliferative properties [7–9]. Also, DOX complexes with transition metals revealed interesting biological activity, as new anticancer drug candidates [10].

In an everyday human diet, plants play a key role as the essential source of substances (vitamins, minerals, enzymes, antioxidants), which are necessary for the proper function of the body. Many of plants are characterized by high biological activity of these substances and are used as drugs or chemopreventive agents in the treatment of some civilization diseases, such as cancer, diabetes and cardiovascular diseases. Epidemiological studies revealed, that selenium (Se) is one of the most extensively studied cancer and cardiovascular disease compound – as preventing and antioxidant element. Se can exist in various oxidation forms, specifically in association with three oxygen atoms (selenite, Se^{4+}), or four oxygene atoms (selenite, Se^{6+}). Selenium in organic forms is bivalent (e.g. selenocysteine or selenomethionine). Selenium exists in human organism in various organic forms, mostly as selenoproteins, the biosynthesis of which utilizes inorganic forms of selenium [11–14]. The primary sources of this element are plants and animals. Selenium, in inorganic form (IV or VI), is absorbed by plants from the soil, and then converted into organic compounds, mainly as selenomethionine and seleno-cysteine and in these forms is consumed by humans. In the human body, it undergoes further transformations, during which it is attached to proteins and amino acids. Se is important in many biochemical and physiological processes including the biosynthesis of coenzyme Q, regulation of ion fluxes across membranes, maintenance of the integrity of keratins, and stimulation of antibody synthesis. This essential trace element has a potential to be used not only in cancer prevention but also in cancer treatment. The antioxidant potency of selenium is associated with its presence in active centers of several antioxidant enzymes, such as glutathione peroxidase (GPx), thioredoxin reductase (TRxR) and iodothyronine deiodinase (DIO), which protect cells from the harmfull effects of free radicals formed during oxidation [11–16]. Selenium in combination with other anticancer drugs or radiation can increase the efficiency of cancer. Recent studies showed apoptosis-enhancement role of selenium in the MCF-7 breast cancer cells treated with DOX. Selenium compounds can induce apoptosis and alternate matrix metalloproteinases expression in malignant gliomas. Selenium co-administrated with doxorubicin protected significantly cardiomyocyte damage in animal model. Moreover, Se can activate some important metabolic enzymes, which are originally controlled through the insulin signal transduction pathway, hence regulating metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway [15,16].

The effectiveness of selenium anti-cancer activity depends on its chemical forms, dose, cancer type and the degree of selenium bioavailability. Not all chemical forms of selenium are equally effective. Only four-valent sodium selenite, but not six-valent selenate, specifically oxidizes the vicinal sulfhydryl groups, thus emphasizing its unique role in the treatment of cancer. Experimental studies suggest, that only selenite (Se^{+4}) prevents the formation of a fibrinogen-albumin coat on the surface of cancer cells. The structure of the fibrinogen-albumin coat is the mechanism of hiding specific antigens on tumor cells before they are recognized by the immune system and NK cells. Moreover, sodium selenite has potency for direct activation of NK cells. Some research revealed, that selenite have an anti-leukemic effect and it can be use as drug for the treatment of multidrug resistant acute myeloid leukemia [11–14].

Vitamin C is primarily involved in collagen biosynthesis, catecholamines and lipid metabolism. Vitamin C is the main antioxidant in blood and body fluids, affects iron absorption and modulates mutagenesis and carcinogenesis, similarly as vitamin E. The daily requirement for vitamin C ranges from 30 to 85 mg. The main sources of vitamin C are fresh fruits and vegetables. Recent studies showed vitamin C dose-dependent antineoplastic activity with influence also on apoptosis, cell cycle, and cell signaling. Some experiments showed synergistic cytotoxic effect of mitoxantrone (antineoplastic agent) and ascorbic acid in breast cancer human cell line [17–20]. Moreover, ascorbate plays an important role in the epigenetic regulation, playing a substantial role in the demethylation of DNA and histone protein. Ascorbate was discovered as a cofactor for methylcytosine dioxygenases that are responsible for DNA demethylation. Additionally, ascorbate can be involved in embryonic development, postnatal development, aging, cancer and other diseases [19,20].

The aim of this study was to investigate the influence of Mn, Mg, Fe, Co and Ni, seleno-L-methionine and vitamin C on biological activity of DOX in prokaryotic model - *Escherichia coli* RFM443. *Escherichia coli* RFM443 contained plasmid transcriptional fusion between *recA* promoter which belongs to DNA-damage genotoxin-inducible group of bacteria promoters, involved in the SOS regulon response. Bioluminescent bacterial sensor is based on the fusion of bacterial bioluminescence gene – *luxCDABE* acting as a reporter element. In this genetic system *recA* promoter was fused to the promoterless *Vibrio fischeri luxCDABE* operon and allowed visualization of the transcriptional responses induced by DNA damage, without the need to perform enzyme assays or to add luciferase substrates exogenously, since the full *lux* operon encodes not only the catalytic luciferase (LuxAB) but also the enzymes required to shunt fatty acyl metabolites from the central metabolism and to convert them to the aldehyde substrate for luciferase [21–25]. Genetically engineered microbial reporter strains are based upon the fusion of an inducible sensing element upstream of a reporting element, so that the construct emits a dose-dependent signal when exposed to the inducing compound(s) or stress factor(s) [23]. As it was previously shown, the above described *E. coli lux* biosensors report the presence of genotoxic doses of stressors by an increase in the production of light and are useful to detect genotoxicants, environmental chemicals, anticancer drugs and some new candidate drugs [26–31].

Cytotoxic properties of tested chemicals were calculated on the basis on growth inhibition (GI) potency of tested chemicals against bacteria culture, measured as optical density (OD) values. Genotoxic potency were calculated on the basis of the dynamic of intensity of luminescence signal emission by bacteria with *lux* gene normalized with control sample and expressed as relative luminescence units (RLU) values.