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Metal transport capabilities of anticancer copper chelators

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ABSTRACT

In the present study, several Cu chelators [2,2′-biquinoline, 8-hydroxiquinoline (oxine), ammonium pyrrolidinedithiocarbamate (APDTC), Dp44mT, dithizone, neocuproine] were used to study Cu uptake, depletion and localization in different cancer cell lines. To better understand the concentration dependent fluctuations in the Cu intracellular metal content and Cu-dependent *in vitro* antiproliferative data, the conditional stability constants of the Cu complex species of the investigated ligands were calculated. Each investigated chelator increased the intracellular Cu content on HT-29 cells causing Cu accumulation depending on the amount of the free Cu(II). Copper accumulation was 159 times higher for Dp44mT compared to the control. Investigating a number of other transition metals, intracellular accumulation of Cd was observed only for two chelators. Intracellular Zn content slightly decreased (*cca.* 10%) for MCF-7 cells, while a dramatic decrease was observed on MDA-MB-231 ones (*cca.* 50%). A similar decrease was observed for HCT-116, while Zn depletion for HT-29 corresponded to *cca.* 20%. The IC_{50} values were registered for the investigated four cell lines at increasing external Cu(II) concentration, namely, MDA-MB-231 cells had the lowest IC_{50} values for Dp44mT ranging between 7 and 35 nM. Thus, Zn depletion could be associated with lower IC_{50} values. Copper depletion was observed for all ligands being less pronounced for Dp44mT and neocuproine. Copper localization and its colocalization with Zn were determined by μ-XRF imaging. Loose correlation (0.57) was observed for the MCF-7 cells independently of the applied chelator. Similarly, a weak correlation (0.47) was observed for HT-29 cells treated with Cu(II) and oxine. Colocalization of Cu and Zn in the nucleus of HT-29 cells was observed for Dp44mT (correlation coefficient of 0.85).

1. Introduction

In the industrialized world, cancer has now become the second most frequent cause of death [1]. Currently a broad range of compounds with different mechanisms of anticancer activity are available for treatment. Several of them could be classified as metal-based drugs. One of the well-known metal based drug is cisplatin [*cis*-diammine-dichloroplatinum(II) complex], which has been used for treatment of various types of human cancer. However, the applicability of cisplatin is limited due to its dose-limiting side effects and inherited or acquired resistance [2]. The number of Pt-based compounds is dynamically increasing aiming at broadening the targets [3,4]. Nevertheless, several other metal complexes are under investigation. Thus, research on alternative metal-based complexes came into the spotlight such as complexes of endogenous metals like copper (Cu) [5,6] assuming that they may be less toxic for normal cells.

An important emerging problem is drug resistance of cancer cells that renders many of the treatments ineffective. Multidrug resistant (MDR) selective compounds have been found to be enriched in structures enabling metal chelation [7]. This discovery pointed out to the importance of anticancer metal chelators.

Control of tumor growth, angiogenesis and metastasis can be achieved by chelating the excess of Cu [5,8,9]. Copper ions show preference for either soft or hard donor atoms depending on its valence. According to the ligand donor atom, Cu complexes can be grouped into derivatives of S-donor systems (thiosemicarbazones, thiosemicarbazides, dithiocarbamates, thioureas and dithiolate); O-donor systems (pyridine N-oxides, phenol analogue to 8-hydroxyquinoline,

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naphthoquinones, carboxylates, triethanolamines, *etc.*); N-donor systems (pyrazoles, pyrazole-pyridine, imidazoles, triazoles, tetrazoles, oxazoles, indoles, *etc.*); Schiff-base systems; phosphine and carbine systems, *etc.* [5]. Several Cu complexes have been tested *in vitro*. To date, thiosemicarbazone, phenanthroline, oxine and dithiocarbamate have been considered as the most effective backbones. The first three scaffolds have been unequivocally proved to be effective in MDR models [7,10].

Among thiosemicarbazones, several promising compounds are known. Generally, their Cu complexes are more effective than the parental molecule. The terminally dimethylated di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT) showed high toxicity *in vitro* and *in vivo* [11,12]. Interestingly, these compounds are capable of Fe coordination. Therefore, they are considered to act *via* a dual mechanism[13]. There is a strong link between the thiosemicarbazone (TSC) backbone and MDR selective toxicity, as exemplified by several isatin-β-thiosemicarbazones including NSC73306 [14].

The 1,10-phenantrolines show antineoplastic properties. Both the Cu(I) and Cu(II) chelates of 2,9-dimethyl-1,10-phenantroline (neocuproine) showed to be a potent cytotoxin against different cell lines *in vitro* and its activity potentiated upon available Cu(II) ions in the medium [15,16]. The lipophilic Cu(I)-specific chelator neocuproine has been frequently used as an inhibitor of Cu-mediated damage in biological systems [17]. Similarly, pyrrolidine dithiocarbamates (PDTCs) show its toxic effect by transporting a redox active metal into the cell [18,19]. Following this study, several authors have demonstrated antitumor activity with Cu complexes of dithiocarbamates [20–23].

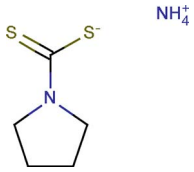
Bis-8-hydroxyquinoline (oxine) Cu(II) was found to inhibit *in vitro* the chymotrypsin-like activity of purified 20S proteasome [24]. When in complex with Cu, oxine inhibited the chymotrypsin-like proteasome activity and induced apoptotic cell death in breast cancer in a dose-dependent manner [25]. Antitumor effects of several metal complexes of oxine have been reported [26]. Moreover, oxine is one of the most promising scaffold for the development of MDR compounds [27].

The mechanism of action and the target(s) of anticancer chelators are unknown. Assuming that chelators act intracellularly through chemical reactions, the mechanism of action may be either based on metal depletion and/or metal accumulation. Several mechanistic approaches for the mechanisms of action of Cu complexes in anticancer therapy have been formulated. Thus, Cu derivatives interact noncovalently with DNA double helix through intercalative, electrostatic and groove binding. The intercalation favors DNA oxidative cleavage. It was observed that Cu complexes (*i.e.*, Cu salicylaldoxime, Cu(II)-TSCs) can inhibit topoisomerases, the latter playing an essential role in DNA replication and transcription [5]. Cancer cells are more sensitive to proteasome inhibition than normal cells. The first Cu chelator for which this effect has been proved was disulfiram belonging to the group of dithiocarbamates [28]. Although many Cu-based antitumor agents have proved to be cytotoxic *in vitro*, their further utility was limited by the poor water solubility and relatively high *in vivo* toxicity [29].

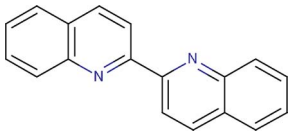
We have previously determined the *in vitro* cytotoxicity on several representatives belonging to the chelator classes of dithiocarbamate, phenantroline, quinoline, thiocarbazone, thiosemicarbazone using different cancer cell lines. The antiproliferative activity was more pronounced in the presence of Cu(II) [30]. These high intracellular Cu concentrations induced reactive oxygen generation and apoptosis in a Cu content dependent manner. However, the DNA damaging effect and the accumulation of Cu chelators into the DNA structure were not found in most cases.

In the lack of a systematic comparison of Cu levels by using chelators as potential antitumor agents, the main objective of the present study was to evaluate the *in vitro* uptake, depletion and localization of Cu. Moreover, Cu-mediated toxicity and interactions with other similar divalent metal ion were also aimed to be studied.

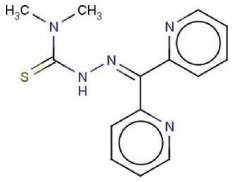
Table 1
Structural formulae of the investigated Cu chelating agents.



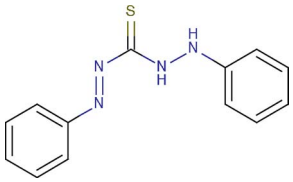
Ammonium pyrrolidinedithiocarbamate **(APDTC)**



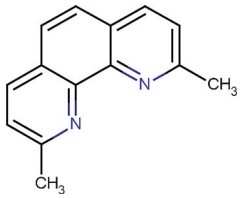
2-quinolin-2-ylquinoline **(2,2′-biquinoline)**



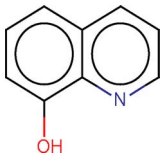
Di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone **(Dp44mT)**



Diphenylthiocarbazone **(dithizone)**



2,9-Dimethyl-1,10-phenanthroline **(neocuproine)**



8-Hydroxyquinoline **(oxine)**

2. Materials and methods

2.1. Copper chelating agents and cell lines

Throughout the experiments, deionized Milli-Q (Millipore, Molsheim, France) water with a relative conductivity of 18.2 MΩ cm was used. All the chemicals were of analytical grade, if not stated otherwise. Copper(II) sulfate and the different chelating agents [2-quinolin-2-ylquinoline (2,2′-biquinoline), oxine, ammonium pyrrolidinedithiocarbamate (APDTC), Dp44mT, diphenylthiocarbazone (dithizone), neocuproine] were purchased from Sigma Aldrich Ltd. (Budapest, Hungary – hereafter Sigma Aldrich). The structural formulae of the investigated ligands can be seen in Table 1. D-Penicillamine and triapine were used as extracellular and intracellular [31] negative controls, respectively.

The following human cancer cell lines were used: HCT-116 and HT-29 colon adenocarcinomas as well as MCF-7 and MDA-MB-231 human breast adenocarcinomas. These cell lines were obtained from ECACC (Salisbury, UK). Human tumor cell line ZR-75-1 was purchased from ATCC (LGC Standards GmbH Wesel, Germany). Concentrated nitric acid (65%) and hydrogen peroxide (30%) of Suprapur quality needed for sample preparation of cell lines were supplied by Merck (Darmstadt, Germany).

2.2. Determination of complex stability constants

The conditional stability constants complexes of Cu(I) and Cu(II) with 2,2′-biquinoline, oxine, APDTC, Dp44mT and neocuproine were determined by UV/V is spectrophotometry according to our previous study [13]. Briefly, a solution with a concentration of 50 μM of each chelator in 0.15 M KNO_3 was titrated with calculated volumes of 3 mM Cu(II) salt solutions. In order to prevent the reoxidation of Cu(I), 10 mM ascorbic acid was also dissolved in the titrant solution. This solution was stable over 24 h. The chemical shift or absorbance versus metal ion concentration data-sets were evaluated by the OPIUM software.