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Photocytotoxicity of [Cu(N-salicyliden-L-glutamato · $2H_2O$] · isoquinoline

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Abstract: The metal complexes have a significant role in photodynamic therapy (PDT). The most common metals studied for PDT of tumors are transitional metals. In this paper we have studied the copper(II) complexes of N-salicyliden-L-glutamate. The photocytostatic effect was monitored against mouse leukemic cell line L1210. Photocytostatic effect was confirmed for [Cu(N-salicyliden-L-glutamato · $2H_2O$] · isoquinoline (CuCIQ), but it wasn't observed for the parental molecule [Cu(N-salicyliden-L-glutamato · $2H_2O$] · H_2O. CuCIQ had photocytotoxic effect already at 10 μM concentration. Irradiated cells (1050 mJ.cm^{-2}, λ = 365 nm) had half the viability of the cells incubated with CuCIQ in the dark (72 h). Co-incubation of the cells with CuCIQ and ascorbic acid increased this photocytotoxic effect and this effect correlated with the elevated level of lipoperoxidation.

Keywords: photodynamic therapy, photosensitizers, metal complexes, ascorbic acid

Introduction

Photodynamic therapy (PDT) is a method for treatment of cancer. This therapy uses photosensitiser and light with suitable wavelength, whereby the destruction of cancer cells is induced by production of reactive oxygen species (Dolmans et al. 2003, Yano et al. 2011). There are several porphyrin-related photosensitizers (PS) approved for clinical usage (O'Connor et al. 2009). However many research groups are studying novel derivatives as potential photosensitizers, including metal-based PS (Josefsen and Boyle 2008). Metals (mostly transition metals) are usually conjugated with macrocyclic PS. Octaethylbenzochlorin photosensitiser metallated by copper showed potent photocytotoxic effect *in vitro* and *in vivo*, for leukemic cells and rat bladder tumor, respectively (Josefsen and Boyle 2008).

Recently, it was found that [Cu(N-salicyliden-L-glutamato · $2H_2O$] · isoquinoline (CuCIQ) and its parental molecule [Cu(N-salicyliden-L-glutamato · $2H_2O$] · H_2O possess antimicrobial and anticancer properties and furthermore they can intercalate

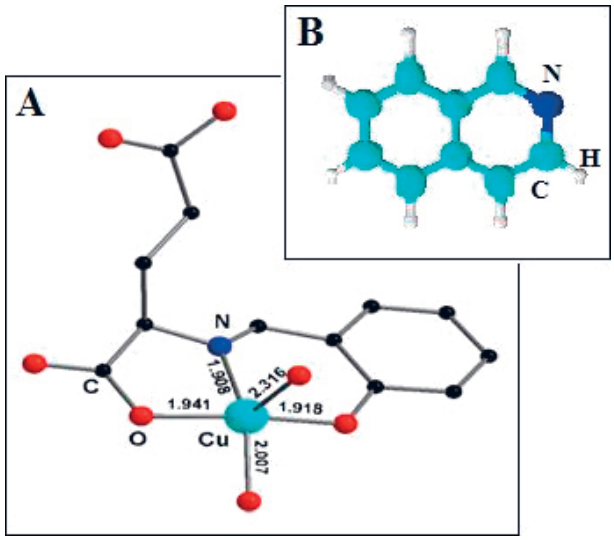

Fig. 1. Structure of [Cu(N-salicyliden-L-glutamato · $2H_2O$] · H_2O (**A** — parental molecule), isoquinoline (**B**) (Sabolová et al. 2011).

DNA (*in vitro* studies). Their antimicrobial effect was enhanced several times after co-treatment with ascorbic acid (Kohutová et al 2007, Paulíková et al. 2008, Sabolová et al. 2011).

In this paper we have studied photocytotoxic effect of [Cu(N-salicyliden-L-glutamato · $2H_2O$] · isoquinoline (CuCIQ) and its parental molecule [Cu(N-salicyliden-L-glutamato · $2H_2O$] · H_2O. Photocytotoxicity and oxidative stress induced by CuCIQ in the cells was modulated by co-treatment with ascorbic acid.

Experimental

Materials and methods

Cell culture

Mouse leukemic cell line L1210 was obtained from Dr. P. Ujhazy, Roswell Park Cancer Institute, Buffalo. L1210 cell line was grown in RPMI 1640 medium supplemented with 10 % FBS, penicillin (100 units/mL), streptomycin (100 μg/mL). The cells were maintained at 37 °C in a humidified 5 % CO_2 atmosphere.

Irradiation of cells

L1210 cells were seeded in Petri dishes, covered with lids, kept on ice and irradiated with UV light (365 nm, UV lamp HB175 PHILIPS). Maximal used light dose was 2100 mJ.cm^{-2} didn't affect the viability of non-treated cells (control). Parallel series of samples were kept in the dark.

Direct cell counting

Cytotoxic potential of compounds was determined by a trypan blue dye exclusion test (direct cell counting) (Altman et al. 1993). Cells were seeded (1 × 105 cells/mL) in Petri dishes and tested substances (or 1 % DMSO-control) were added after 24 h. Cell viability was checked after 24, 48 and 72 h. All dye exclusion tests were made three times.

Analysis of cytomorphology

L1210 cells were seeded (0.25 ~ 10^6 cells/mL) and treated with 20 μM copper(II) complex without or in the presence of ascorbic acid (100 μM). Final concentration of DMSO in the samples was 1 %. After treatment (24 and 48 h), the cells were washed in PBS and cytomorphology was observed using light microscope (Axio Zeiss Imager A1, camera AxioCam MRc) (magnification 63 × 10).

Detection of lipoperoxidation

L1210 cells were diluted (7 × 10^6 cells/mL) and washed with PBS. Samples were irradiated (2100 mJ.cm^{-1}) after 30 min incubation with CuCIQ (10 μM) without or in the presence of ascorbic acid (100 μM). The level of MDA was determined after reaction with thiobarbituric acid (TBARs) according to Sturlan et al. (2003) (ε_{532} = 157 mM^{-1}cm^{-1}).

Results and discussion

Photocytotoxic effect of CuCIQ against leukemic cell line L1210 was monitored during the 3-day

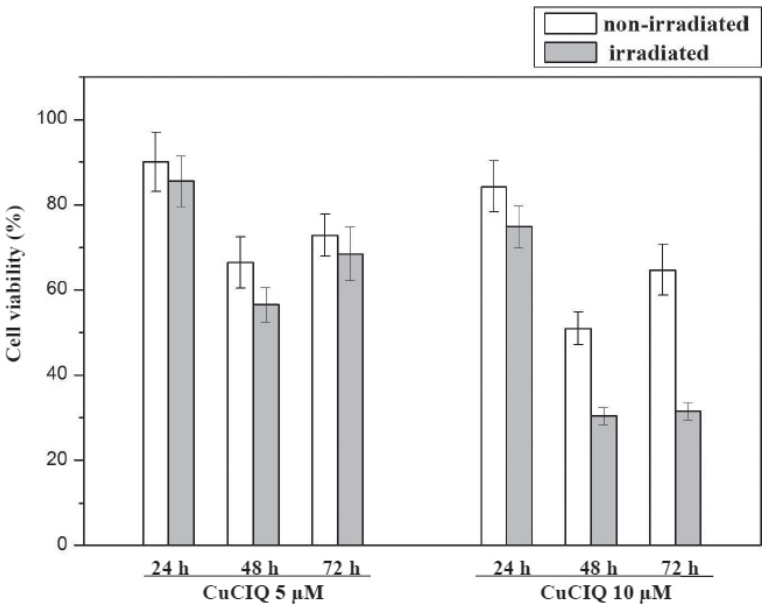

Fig. 2. Photocytotoxic effect of CuCIQ against L1210 cells. The cells were treated with CuCIQ (5, 10 μM) and the cell viability (without and after irradiation (1050 mJ.cm^{-2}, λ = 365 nm)) was evaluated after 24 h, 48 h and 72 h.

cultivation. As seen in Fig. 2, there wasn't a significant difference between viability of irradiated and non-irradiated samples after 24 h incubation. Increase in CuCIQ cytotoxicity after irradiation was observed after long-term incubation (72 h). 10 μM CuCIQ decreased the viability of cells without and after irradiation to 65 % and 30 %, respectively. As seen in Fig. 2 cytotoxic effect of 5 μM CuCIQ wasn't permanent, and the cells seem to adapt to oxidative stress.

Contrary to expectations the photocytotoxic effect of parental molecule was not observed, the cytotoxicity of 20 μM complex didn't increase after irradiation (1050 mJ.cm^{-2}, λ = 365 nm) of the cells (data not shown). According to our results, photocytotoxicity of CuCIQ is closely connected to the isoquinoline ligand in the complex. Previously it was shown, that isoquinoline itself was not cytotoxic but binding of parental molecule with ligand enhanced cytotoxicity of CuCIQ (Vaľková 2004).

Sabolová et al. (2011) have found that combination of CuCIQ with antioxidant — ascorbic acid caused elevation of production of free oxygen radicals (ROS) in yeast cells. ROS can damage biological macromolecules, including lipids. Therefore we monitored the level of the end product of lipid peroxidation — malondialdehyde (MDA) in the cells. L1210 cells incubated with CuCIQ (10 μM) and together without or with ascorbic acid were irradiated (2100 mJ.cm^{-1}). The level of TBARs is presented in Fig. 3.

Lipoperoxidation was markedly increased after adding ascorbic acid. Pro-oxidant effect of ascorbic acid in the presence of Cu ions was observed. Membrane fluidity can be altered due to lipoperoxidation (de la Haba et al. 2013) and changes in ion permeability can occur. Although the transport of the Cu-complexes into cells has not been studied we suppose that CuCIQ is not able to enter into cells. Cellular uptake and efflux of metal complexes is not understood well. Price et al. (2011) have found that copper bis(thiosemicarbazonato) complexes may be taken into the cells by combined passive and facilitated (protein-carrier-mediated) mechanisms. But, they also demonstrated that copper from copper(II) complexes was rapidly effluxed from the cells through active mechanisms. Perturbation of membrane fluidity can affect CuCIQ influx and its cytotoxicity can elevate. We monitored whether adding ascorbic acid escalates photocytotoxicity of CuCIQ. L1210 cells incubated with CuCIQ (20 μM) and ascorbic acid (100 μM) were irradiated and viability was determined (24 h). Photocytotoxicity of CuCIQ increased (Fig. 4) and viability of cells in co-treatment was reduced by 10 %. The combination of CuCIQ and ascorbic acid scaled its antimicrobial effect up several times (Sabolová et al. 2011).

Seeing that CuCIQ possesses photocytotoxicity against L1210 we monitored cytomorphologic changes induced by the complex. Observing cytomorphology was the first step to determine the type of cell death. L1210 cells treated with CuCIQ (20 μM) and in combination with ascorbic acid (100 μM) were irradiated (1050 mJ.cm–2) and observed by light microscopy (24 h and 48 h incubation). As shown in Fig. 5, the cells treated with CuCIQ shrank their volume (Fig. 5), but they

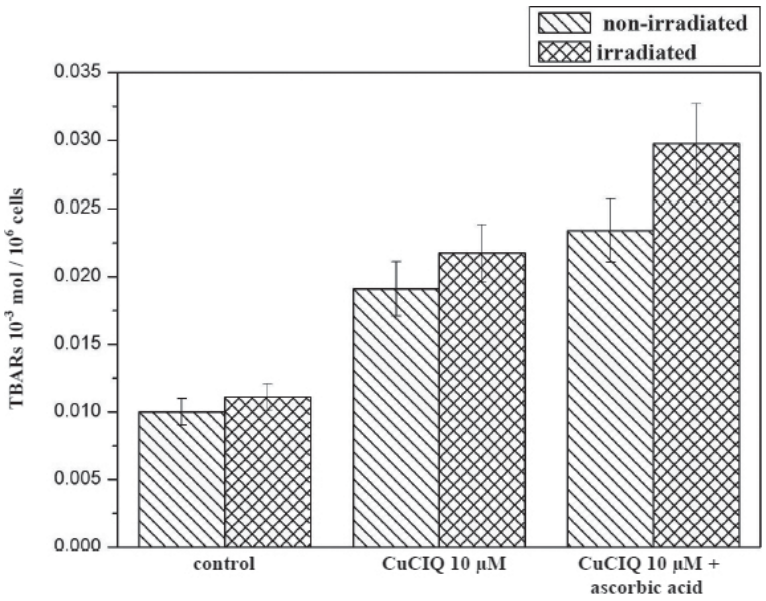

Fig. 3. The level of TBARs in non-irradiated/irradiated (2100 mJ.cm^{-2}) cells after incubation with CuCIQ (10 μM) without or with ascorbic acid (100 μM).

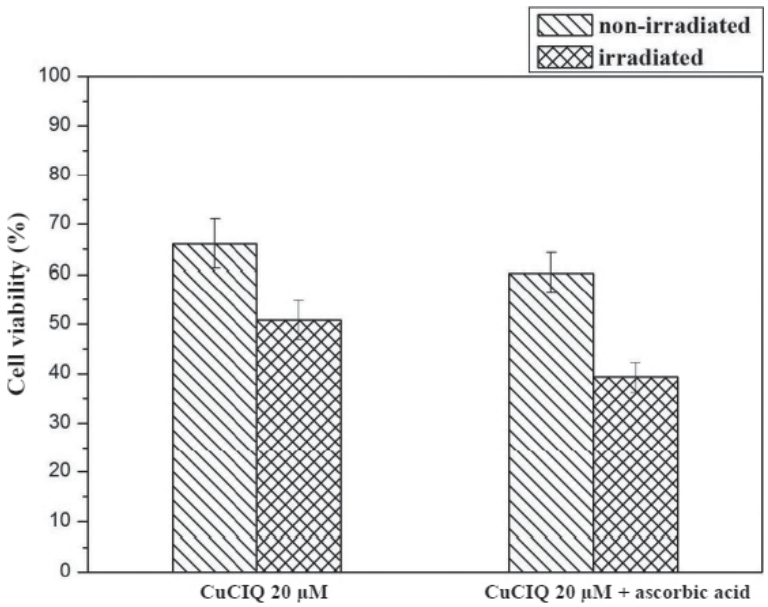

Fig. 4. Increase in CuCIQ photocytotoxicity against L1210 cells by co-incubation with ascorbic acid. Cells were treated with CuCIQ (20 μM) without or with ascorbic acid (100 μM).

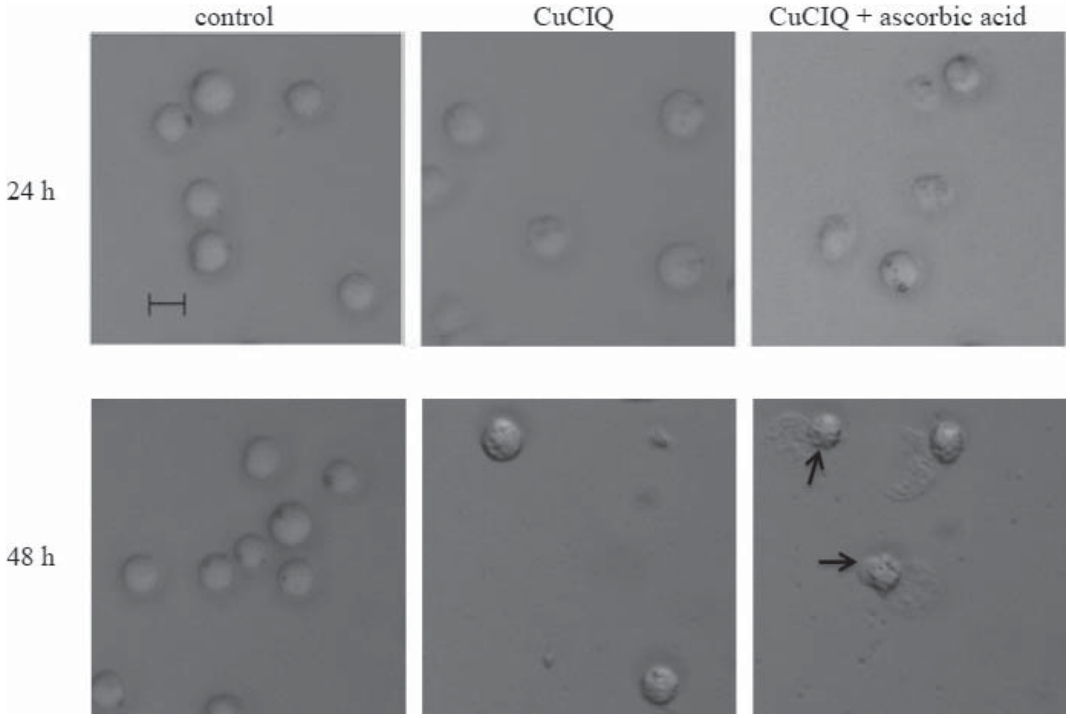

Fig. 5. Cytomorphologic changes of L1210 induced by CuCIQ and irradiation. Cells were treated with CuCIQ (20 μM) or CuCIQ with ascorbic acid (100 μM), irradiated (1050 mJ.cm^{-2}) and monitored by light microscopy (24 h and 48 h). Arrow shows cell shrinkage, bar represents 10 μm.

kept their rounded shape. Combined treatment of CuCIQ with ascorbic acid against L1210 cells caused the loss of cell shape (Fig. 5) and cell shrinkage was observed. These changes are in accordance with the changes during apoptosis but further studies are needed to confirm it.

Conclusion

[Cu(N-salicyliden-L-glutamato · 2H_2O] · isoquinoline (CuCIQ) is copper(II) containing complex with anticancer activity against leukemia cells L1210. Photocytotoxic effect of CuCIQ was confirmed after

irradiation of the cells (1050 mJ.cm^{-2}, λ = 365 nm). Combined treatment of L1210 cells with CuCIQ and ascorbic acid elevated oxidative stress in the cells therefore also photocytotoxic effect of the combined treatment was more effective. The leukemic cells died probably through an apoptotic process (the combined treatment: CuCIQ, ascorbic acid and irradiation), which indicates that the complex can be a promising drug for cancer.

Acknowledgments

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