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EFFECTS OF BINDING METRONIDAZOLE TO A COPPER-ACETATE COMPOUND ON RADIOSENSITIZER PROPERTIES

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ABSTRACT

Copper compounds exhibit interesting biological properties. Nitroimidazoles show radiosensitizer properties for radiotherapy tumor treatment. In the present work, the effect of binding metronidazole (1-(2-hidroxyethyl)-2-methyl-5-nitroimidazole = MTZ) to copper-acetate on the radiosensitizer properties has been investigated. A compound of copper-acetate-MTZ was prepared and characterized. The experiments were carried out by gamma-irradiation of Hep2 (human larynx cancer) cells under hypoxic conditions. The radiation doses for 50% cell survival in the presence of radiosensitizer were about 8.2 Gy for CuAcMTZ or free MTZ. The effect of binding metronidazole to copper acetate on radiosensitizer properties is mainly related to the radiosensitizer process which involves two events for CuAcMTZ in contrast to one event observed for the MTZ free drug.

1. INTRODUCTION

Transition metal compounds are able to enhance cellular radiation damage both *in vitro* and *in vivo* [1] and have been proposed as radiosensitizers for tumors cells. A metronidazole-containing Cu(II) compound has been investigated as a possible alternative to the metronidazole (MTZ) organic radiosensitizer. Although MTZ sensitizes efficiently hypoxic cells to damage induced by γ-radiation, its use for radiation therapy of cancer cells is limited by the toxicity in the clinical doses required for radiosensitization. Studies on gamma radiolysis and radiosensitization of tymine by a copper-acetate-MTZ (CuAcMTZ) compound have been reported [2-3]. However, no studies *in vitro* involving hypoxic cells have been found for this compound. In the present work, the CuAcMTZ has been prepared by a modified method and its radiosensitizer property has been investigated for Hep2 (human larynx cancer) cells and compared to that of the MTZ organic compound.

2. MATERIALS AND METHODS

2.1. Synthesis of the compound CuAcMTZ

Hot ethanolic solutions of copper acetate (0.40 g/30 mL) and MTZ (0.60 g/20 mL) were mixed and stirred at room temperature for two hours. Then, the volume of the solvent was reduced to 10 mL and the solid was filtered off, washed with ethanol and dried *in vacuo*. Yield: 60%. Anal. calcd. for $Cu_2C_{20}H_{30}N_6O_{14}$: C 34.0; H 4.3; N 11.9; found: C 34.3; H 4.1; N 12.0 %. UV-VIS, λ (nm): 309, 702. FTIR main bands, ν(cm^{-1}): 1621, ν_a(COO); 1432, ν_s(COO); 1542, ν(C=N).

2.2. Determination of radiation lethal dose (rLD_{50})

Experiments were conducted *in vitro* under anaerobic conditions for Hep2 cell line (human larynx cancer cell of American Type Culture Collection [ATCC-CCL23)]) by adapted neutral red uptake methodology [4]. The cells were mantained in MEM (Minimum Eagle´s Medium with 10% fetal calf serum, 0.1 m mol/L non-essential amino acids and 1 mmol/L sodium piruvate). The cells were detached with 0.2% trypsin and 0.02% EDTA and washed. A suspension of about 1.0 x 10^5 cells/mL of culture medium was seeded in each well of 96 wells microplate and incubated (INC: incubation under humidified air atmosphere, 5% CO_2, at 37 ^oC) for 24 h. After that, culture medium was changed by fresh MEM and the microplate was introduced into partially opened plastic boxes and enclosed into plastic bags under microaerophilic conditions created by using CampyGen sachets (Oxoid). Then, the samples were exposed to a ^{60}Co γ-rays from a panoramic radiation source with dose rate of 0.89 Gy min^{-1} for periods of time correspondent to doses of 0; 50 and 100 Gy. Culture medium were changed again and the samples were incubated (INC) for 24 h. Then, the culture medium was replaced by a solution of neutral red dye-containing MEM (50 μg/mL). After incubation (INC) for 3 h, the microplates were washed with phosphate buffer (PBS) and a solution of 1% $CaCl_2$ in 0.5% formaldehyde. Each well received 0.2 mL of 1% acetic acid in 50% ethanol and the optical densities (OD) were measured at 540 nm in an ELISA reader spectrophotometer Sunrise from Tecan. The cell viability was calculated in relation to control cell, non-irradiated microplate (zero Gy = 100%).

2.3. Radiosensitizer assay

The procedure was similar to that described for determination of rLD_{50}. The microplate preparation was the same, however, before the radiation process the microplate culture medium was replaced by radiosensitizer-containing MEM, 0.2 mL in a concentration of 100 μmol/L each well. The samples were irradiated in anaerobic conditons for periods of time correspondents to doses of 0; 3; 6 and 10 Gy. After irradiation the radiosensitizer solution was replaced by fresh MEM and the microplate was incubated (INC) for 24 h and the methodology continue as rLD_{50}.

2. RESULTS AND DISCUSSION

3.1. The compound CuAcMTZ

The synthetic method proposed here is based on the solvent ethanol showing advantages in relation to the toxic methanol that has been used before to prepare this compound [5-6]. CuAcMTZ is a dimer of formula $[Cu_2(O_2CCH_3)_4(MTZ)_2]$ which exhibits paddle-wheel structure where four equatorial acetato ligands bridge two Cu(II) ions and two axial MTZ molecules are bounded to Cu(II) by *N*-imidazoles (Fig.1).

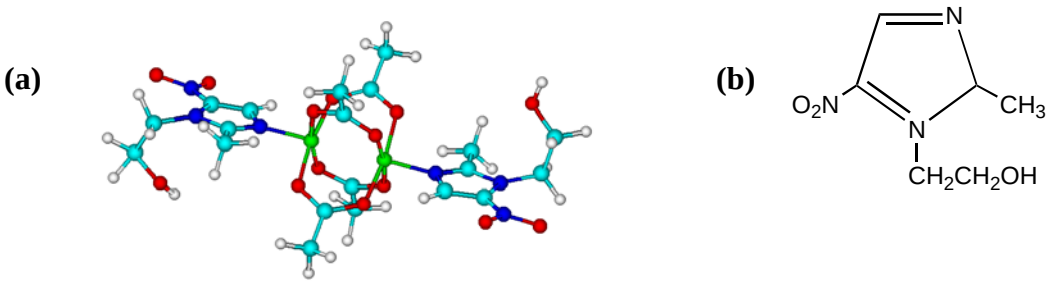

Figure 1. Molecular structure of CuAcMTZ (a); MTZ, [1-(2-hydroxyethyl)-2-methyl-5-nitro-1*H*-imidazole] (b).

3.2. Radiation lethal dose (rLD_{50})

The radiation lethal dose, *i.e.*, the radiation dose that kills 50% cellular population, was determined based on the plot of cell viability logarithms in function of radiation doses (0; 50 and 100 Gy). The value of rLD_{50} found for Hep2 cells was 34 Gy (Fig. 2).

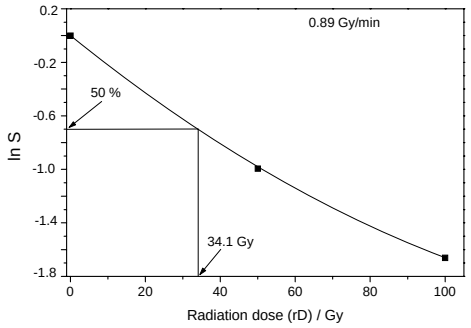

Figure 2. Survival curves for Hep2 cells – determination of rLD_{50}.

3.3. Radiosensitizer properties of CuAcMTZ and MTZ

Chemical agents are called radiosensitizers when increases the hypoxic cells sensibility to radiation in order to improve the cancer radiotherapy treatment efficiency.

Radiosensitizer properties of CuAcMTZ and MTZ have been evaluated based on experimental data for radiation doses of 0; 3; 6 and 10 Gy. The curves of survival fraction (S) logarithms in function of radiation doses (Fig. 3) were adjusted to a quadratic linear model [7]:

$$\ln S = - \alpha D - \beta D^2 \qquad (1)$$

where S is the survival fraction, *i.e.*, the ratio between the number of survival cells for a determined radiation dose and the number of survival cells for a non-irradiated sample; D is the radiation dose; α and β are constants that correspond, respectively, to one event and two independent events produced by radiation.

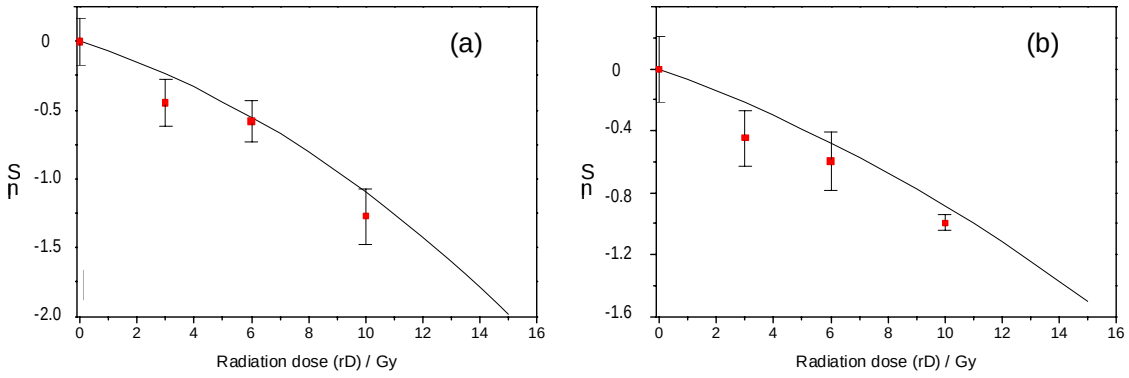

Figure 3. Survival curves for Hep 2 cells in the presence of 100µM/mL CuAcMTZ (a) and MTZ (b).

The model proposed by Chapman *et al.* [7] was used to estimate the type of event for the radiosensitizer processes. Results showed that the process for MTZ free drug (α = 66 x 10^{-3} Gy^{-1}; β = 48 x 10^{-3} Gy^{-1}) occurs by one event whereas the process for CuAcMTZ (α = 65 x 10^{-3} Gy^{-1}; β = 67 x 10^{-3} Gy^{-1}) involves two independent events.

The values of radiation doses correspondent to survival percentages of 10% (S=0.1), 40% (S=0.4) and 80% (S=0.8) are shown in Table 1 together with ER (enhancement ratios).

Table 1 show that the values of rD for CuAcMTZ are lower than those observed for the MTZ free drug. The differences on radiation doses for CuAcMTZ and MTZ are more pronounced for lowest survival fractions. Significant radiosensitizer properties (ER > 1) are observed for S=0.8 for both of compounds.

Table 1. Radiation doses (rD) and ER* for S= 0.1; 0.4 and 0.8.

Compound	$rD_{0.1}$	$ER_{0.1}$	$rD_{0.4}$	$ER_{0.4}$	$rD_{0.8}$	$ER_{0.8}$
CuAcMTZ	16.5	0.8	8.8	1.1	2.8	2.2
MTZ	20.4	0.7	10.3	0.9	3.0	2.0

* ER = rD_S^o /rD_S; rD_S^o = radiation dose without radiosensitizer, rD_S = radiation dose in the presence of radiosensitizer.

The radiation doses that give 50% cell survival (S=0.5) for Hep 2 cells in hypoxic condition and in the presence of radiosensitizer was about 8.19 Gy for CuAcMTZ and 8.23 Gy for MTZ. These doses are four times lower than the rLD50 (34Gy). Therefore, the results indicate that both of the studied compounds exhibit radiosensitizer properties, increasing the gamma-irradiation effect for the same cell culture mortality.

Data showed above indicate that the radiation dose for S=0.5 was about the same (8.2 Gy) for Hep 2 cells in the presence of similar concentrations (100 μmol L^{-1}) of both radiosensitizers (CuAcMTZ and MTZ). However, each molecule of CuAcMTZ compound carries two molecules of MTZ, meaning that 100 μmol L^{-1} CuAcMTZ would correspond to 200 μmol L^{-1} MTZ. Moreover, the experiments indicate that the radiosensitizer processes do not involve the same number of events for copper-bounded MTZ and free MTZ. Based on these results it is possible to propose that binding MTZ to copper acetate modifies its radiosensitizer properties.

3. CONCLUSIONS

The CuAcMTZ compound exhibts radiosensitizer property increasing the gamma-irradiation effect for Hep2 cells mortality. The effect of binding metronidazole to copper acetate on radiosensitizer properties is mainly related to the radiosensitizer process which involves two events for CuAcMTZ in contrast to one event observed for the MTZ free drug.

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