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Stability, Toxicity and Cytotoxicity of a Cupric Complex Towards Cultured CaCo-2 Cells

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Abstract. The human colon adenocarcinoma-derived cell line CaCo-2 was used as a model system to study the effects of copper (II), tetradentate N-alkyl ligand (H₂L) and their complex. The stimulatory effect of the complex was obtained with lower concentrations from 10⁻⁷ to 10⁻¹⁷ mol.L⁻¹, while inhibiting effects occur from 10⁻⁴ to 10⁻⁶ mol.L⁻¹. According to this study, copper (II) and free ligand were toxic for cultured cells. Our data suggested that the complex, according to our toxicity assays in mice, could be used as an antimetabolic agent.

We recently described the potent cytotoxicity of the complex [Cu(H₂L)(H₂O)₂]Cl₂·4H₂O (H₂L=N,N'-bis(2-hydroxyacetophenyl)-1,2-diaminoethane) towards various leukemia, lymphoma and carcinoma cells [1]. We have demonstrated that this complex causes a 10 % reduction in DNA topoisomerase II inhibition at 150 μM. In an attempt to obtain metallic complexes with the aim of enhancing copper (II) effects against cancer with less toxicity, we have already studied in mice, the toxic effects of metallic complexes obtained with cupric ions and 3-(2-furyl) prop-2 enal semicarbazone [2]. In our continuous investigations concerning new antimetabolic metal complexes, we have described the stability *versus* time of the copper salt (CuCl₂), the free linear tetradentate ligand and their complex [3]. We also determined and compared their cytotoxicity in cultured CaCo-2 cells and their toxicity in male Swiss mice.

Materials and Methods

Chemicals. The synthesis and the chemical characterization of the ligand (Figure 1) N,N'-bis(2-hydroxyacetophenyl)-1,2-diaminoethane (H₂L)

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and the complex [Cu(H₂L)(H₂O)₂]Cl₂·4H₂O have been previously described [3]. Copper (II) chloride CuCl₂·2H₂O of analytical grade was purchased from Fluka and used without further treatment.

The HPLC chromatograms were recorded using Rosorb OD315 C8 column with Gilson and Sopares pumps and Gilson autosampling injector; the detector was UV-Spectromonitor D (LDC/Milton Roy) at 210 nm. Dulbecco's modified Eagle essential medium (DMEM) was from Jacques Buy, France. Other biological chemicals were from Eurobio, France.

Cells cultures. CaCo-2 cells, from passage 75 to 100 were routinely cultured in a culture flask at 37 °C in a humid atmosphere obtained with 90 % of O₂ and 10 % of CO₂ in DMEM containing 20 % foetal calf serum, 1 % penicillin-streptomycin and 1 % non essential aminoacids (AANE). The cultures were fed every 48 hours.

The medium was changed and new treatments were added to the cell cultures every 48 hours. The tested molecules were added to the cultures in aqueous solutions. The cells were trypsinized and counted using an hemocytometer.

Cytotoxic activities. To perform cytotoxicity activity, cells were plated in 96-well tissue culture plates (10¹⁰ by cm²). After overnight growth, the medium was changed to DMEM with studied molecules at various concentrations. Cells were washed with phosphate buffer saline (PBS), fixed with 20 % (V/V) trichloroacetic acid and the plates were washed with PBS five times and then dried for 30 minutes. Sulforhodamin B (SRB) in acetic acid (0.04 % w/w) was added over 30 minutes. The plates were rinsed three times with 1 % acetic acid. SRB was extracted with tris-base buffer at pH = 10.4. The incorporation of SRB to the cells depended on protein synthesis.

Toxicity test. Male Swiss mice from Debré (France) weighing from 20 to 25 g were used because of their regular weight gain and the absence of oestral cycle with changes in sexual hormonal status, which could lead to some errors in *in vivo* experiments. The animals had free access to both water and a standard diet containing 0.8 % Ca, 0.7 % P and 3 UI.g⁻¹ vitamin D. They were placed at room temperature (20 ± 2 °C) and exposed to a 12 hour light / 12 hour dark cycle. After being injected with various doses of studied complexes, the animals were kept under the same conditions and were followed for 7 days.

Results

Complex stability. The HPLC chromatograms of all complexes, are presented in Figure 2 and Table I summarizes

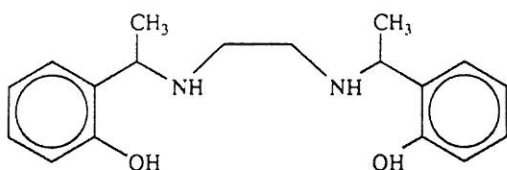


Figure 1. Chemical structure of the ligand.

the results of the study of the stability of the complex when stored respectively at 4°C and 37°C. As the retention time and the peak area remained quite unchanged at 4°C as well as at 37°C, the complex was stable for at least a week and could be used for biological tests.

Cytotoxic tests. The main results are presented in Table II. It may be noted here that mitosis of cultured CaCo-2 cells was inhibited by concentrations higher than 10^{-8} mol.L⁻¹ (CuCl₂), 10^{-6} mol.L⁻¹ (ligand) and 10^{-6} mol.L⁻¹ (complex).

Toxicity assays. 1 - copper chloride: The effects of CuCl₂ on male Swiss mice showed that this salt is a very toxic compound. The lethal dose 0 (LD 0) was 5µg.kg⁻¹ body weight (BW); LD 25 was 15 µg.kg⁻¹ BW; LD 50: 25 µg.kg⁻¹ BW; LD 75: 35 µg.kg⁻¹ BW and finally LD 100 was 45µg.kg⁻¹ BW.

2 - ligand: A dose up to 45µg.kg⁻¹ BW, did not kill any mice 7 days after the injection.

Table I. Retention times (RT) and peak areas (PA) for the stability study of the complex.

Time	4 °C		37 °C	
	RT (min)	PA	RT (min)	PA
9 h	8.44	1005	8.44	951
11 h	8.33	952	8.12	1003
13 h	8.15	1003	8.47	951
15 h	8.75	1017	8.92	985
17 h	9.05	1027	9.05	990
24 h	8.71	1042	8.30	951
2 days	8.54	1015	8.42	983
3 days	8.13	965	7.92	895
4 days	7.94	899	8.47	961
5 days	8.47	955	8.69	1021
6 days	8.74	1069	8.55	875
7 days	8.00	875	8.12	865

3 - complex: The results obtained were the same as with the ligand.

Discussion

According to our results, the complex [Cu(H₂L)(H₂O)₂]Cl₂.

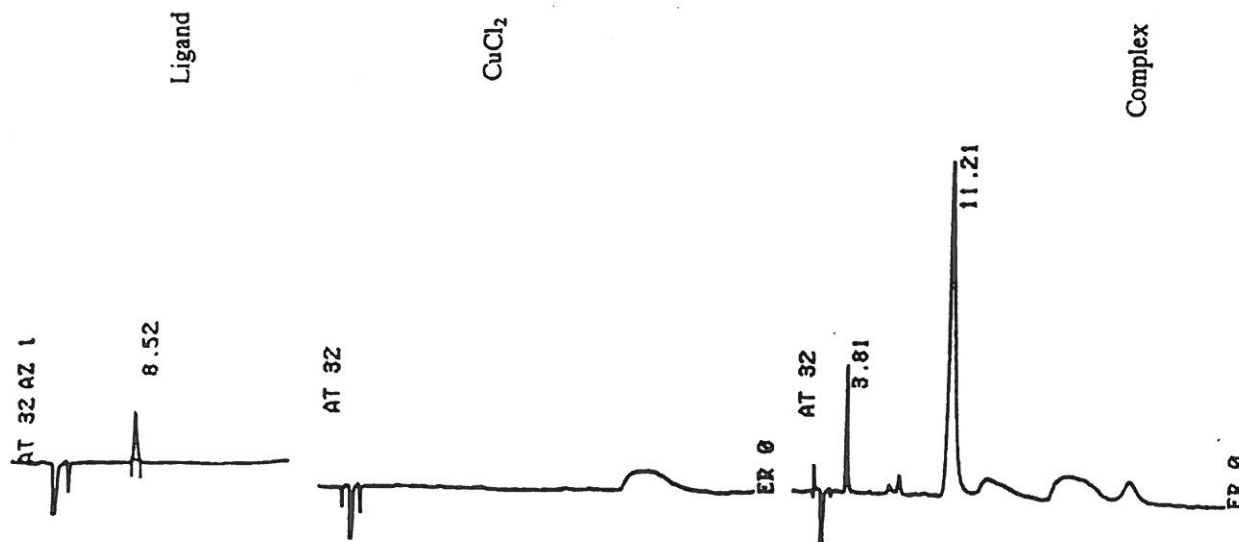


Figure 2. HPLC chromatograms.

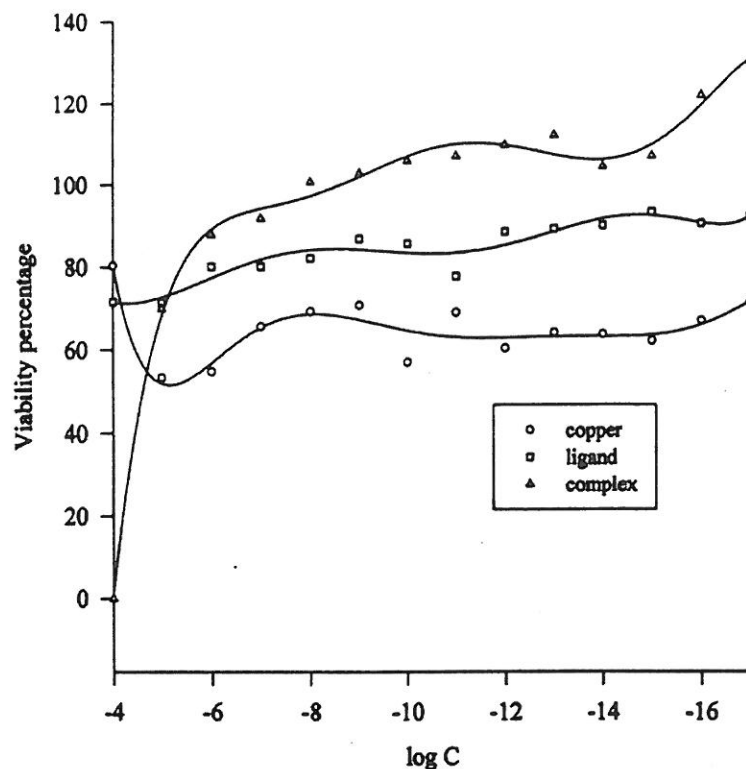


Figure 3. Viability percentages versus concentration logarithm.

$4\text{H}_2\text{O}$ was stable at 4°C and 37°C for 7 days. Retention times and peak areas remained quite unchanged (Table I and Figure 2). This is a very important result as the CaCo-2 cells were cultured at 37°C for at least 48 hours with copper (II) chloride, ligand and complex dissolved in water and then stored at 4°C .

We already tested cobalt (II) cation as anticancer treatment and some interesting results were obtained [4, 5]. The copper (II) cation is the main cofactor for many enzymatic systems and has properties against oncogenesis by protecting from free radical effects [6]. Unfortunately, cupric chloride is toxic by itself and cannot be used as an anticancerogenesis. CaCo-2 cells are a cell line derived from human colon adenocarcinoma of malignant origin [7] and the complex $[\text{Cu}(\text{H}_2\text{L})(\text{H}_2\text{O})_2]\text{Cl}_2 \cdot 4\text{H}_2\text{O}$ was used to control colorectal cancer cell growth.

Figure 3 shows that the ligand and copper (II) inhibited cell proliferation from 10^{-4} to 10^{-17} mol.L $^{-1}$, while the complex stimulated cancer cultured cell mitosis in the range 10^{-7} - 10^{-17} mol.L $^{-1}$ and inhibited cell proliferation from 10^{-4} to 10^{-6} mol.L $^{-1}$.

These facts suggest that the complex is less toxic than the free ligand and CuCl_2 in cultured cells. The observations reported in the present study corroborate our previous work in mice [5]. Copper (II) has a LD100 of up to $44 \mu\text{g}\cdot\text{kg}^{-1}$ (body weight) while the ligand and its complex do not kill any animal at this dose. Finally, we can assume that the complex

Table II. Viability percentage of CaCo-2 cells (mean \pm s.d.)

Concentration (mol.L $^{-1}$)	Copper (II) chloride	Ligand	Complex
10^{-4}	73 ± 5	71 ± 6	2 ± 1
10^{-5}	85 ± 7	97 ± 8	70 ± 14
10^{-6}	85 ± 3	105 ± 4	99 ± 9
10^{-7}	90 ± 10	104 ± 9	98 ± 11
10^{-8}	104 ± 16	104 ± 4	101 ± 14
10^{-9}	102 ± 10	106 ± 4	102 ± 16
10^{-10}	106 ± 7	111 ± 40	101 ± 8
10^{-11}	143 ± 22	157 ± 40	112 ± 28
10^{-12}	161 ± 17	186 ± 25	138 ± 19
10^{-13}	155 ± 31	135 ± 11	133 ± 17
10^{-14}	161 ± 16	128 ± 8	136 ± 9
10^{-15}	140 ± 20	168 ± 24	135 ± 11
10^{-16}	121 ± 9	160 ± 21	144 ± 26
10^{-17}	144 ± 8	141 ± 28	147 ± 14

$[Cu(H_2L)(H_2O)_2]Cl_2 \cdot 4H_2O$ ($H_2L = N,N'$ -bis(2-hydroxyacetophenyl)-1,2-diaminoethane) can be used to inhibit CaCo-2 cell growth because of its lower toxicity.

For further experiments, we must take in to account these data and deal with mice presenting induced colorectal cancer. Studies must be done with intact mice as control and the effects of copper (II) chloride, free ligand and complex must be followed for some months after intraperitoneal injection and/or oral administration.

References

- 1 Hall IH, Taylor K, Miller MC, Do thanh X, Khan MA and Bouet GM: Cytotoxicity of copper complexes of 2-furaldehyde oxime derivatives in murine and human tissue cultured cell lines, *Anticancer Res* 17: 2411-2418, 1997.
- 2 Jamali B, Ibrahim G, Bouet G, Khan MA, Allain P and Do Thanh X: Spring time an Autumn time toxic effects of copper, II ,3-(2-furyl prop-2-enal semicarbazone and $[CuCl_2(FASC_2)]$ complex in mice, *Biol Rhythm Res* 29: 229-236, 1998.
- 3 Djebbar-Sid S, Benali-Baitich O and Deloume JP: Synthesis, characterization and electrochemical behaviour of some copper, II complexes with linear and tripodal tetradentate ligands derived from Schiff bases. *Polyhedron* 16(13): 2175-2182, 1997.
- 4 Do Thanh X, Coquin AS, Saïd M, Khan MA, Bouet G, Spring time and fall time effects of 1,25-dihydroxyvitamin D₃, cobalt, II and $[CoCl_2(1,25-OH_2D_3)]$ complex on renal and cerebral enzymatic markers in rats, *Biol Rhythm Res* 27: 1-8, 1996.
- 5 Hall IH, Lackey CB, Kistler TD, Durham RW, Jouad, EM, Khan MA, Do Thanh X, Djebbar-Sid S, Benali-Baitich O and Bouet G: *Die Pharmazie*, in press, 2000.
- 6 Hall IH, Chen SY, Rajendran KG and West DX: The antiinflammatory activity of metal complexes of heterocyclic thiosemicarbazones, 2-substituted pyridine N-oxides and 2-pyridylthioureas. *Appl Organomet Chem* 10: 485-493, 1996.
- 7 Tong WM, Kallay E, Hofer H, Hulla W, Manhart T, Peterlik M and Cross HS: Growth regulation of human colon cancer cells by epidermal growth factor and 1,25-dihydroxyvitamin D₃. *Eur J Cancer* 34: 2119-2125, 1998.

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