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Nickle(II) and cobalt(II) complexes of hydroxyl-substituted triazamacrocyclic ligand as potential antitumor agents

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Abstract—The stability constants for the formation of nickle(II) and cobalt(II) complexes of the ligand [1,4,7]triazecan-9-ol (**L**) were presented. Antitumor activity of two complexes was reported. Nuclei of [Ni**L**]-stimulated BEL-7402 cells clearly exhibited condensation and break down into chromatin clumps typical of apoptosis. Also it exhibited perturbation effects to cell cycle, and optimal induction of apoptosis was found by Flow-Cytometric analysis. But Co**L** complex did not exhibit introduction effects to BEL-7402 cells apoptosis; and could not perturb cell cycle. Ni**L** and Cu**L** complexes could cleave supercoiled DNA (pBR 322 DNA) to nicked and linear DNA, and DNA of cells treated with Ni**L** or Cu**L** complex was obviously damaged; while Co**L** complex only could cleave supercoiled DNA (pBR 322 DNA) to nicked DNA, and DNA of cells treated with Co**L** complex had no significant difference with control.

Polyamines are essential for life.[1] As a result, many studies have targeted polyamine as a potential site for chemotherapeutic intervention.[2] Macrocyclic complexes with a tetraazamacrocyclic ligand (e.g., cyclen, cyclam, and bicyclam) and their derivatives have found utility in antitumor[3] and anti-HIV[4] applications. Over the past decade, many studies have been focused upon metal complexes of cyclic triamines which cleaving carboxyester,[5] phosphoeaster,[6] RNA,[7] DNA,[8] dipeptides and proteins.[9] To our knowledge, few papers published for the cytotoxic properties and the in vivo antitumor effects of triazacyclic polyamines metal complexes. However, recently, we discovered the first cell apoptosis induced by triazacyclic copper(II) complex.[10] We are interested in metal complexes of the ligand **L** as new antitumor regents and as host for transition metals. As such, we have studied the interaction between nickle(II) or cobalt(II) ion with the ligand **L** in aqueous solution. Further, a comparison of their antitumor activity was made. To our surprise, cobalt(II) complex of the ligand **L** could not induce apoptosis of BEL-7402 cells; nickle(II) complex of the ligand **L** could induce apoptosis of BEL-7402 cells, but also optimal induction of apoptosis was found by flow-cytometric analysis. Herein, we report our preliminary results.

Ligand **L** (Fig. 1) was prepared as described earlier.[11] The metal complex for our study was obtained by adding an appropriate amount of a solution of metal ion, to a solution of the ligand. The M:**L** molar ratio was 1:1 for the system M-**L**. The stability constants for the formation of nickle(II) and cobalt(II) complexes of the ligand [1,4,7]triazecan-9-ol (**L**) determined at 298.0 K in 0.1 $mol \cdot L^{-1}$ KNO_3 were shown in Table 1. Some relevant distribution diagrams were shown in Figure 2.

The ligand **L** formed the mononuclear complexes $[MLH]^{3+}$, $[ML]^{2+}$ and $[MLH_{-1}]^{+}$ while no binuclear complexes have been detected in both systems. The stability constant for the species $[NiL]^{2+}$ ($\log\beta_{[NiL]} = 11.09$) is high in comparison with related $[CoL]^{2+}$ ($\log\beta_{[CoL]} = 8.93$) species. For hydroxyl-substituted [10]aneN_3 com-

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plexes the normal (Irving–Williams)[12] order of stability Cu(II)[10] > Ni(II) > Co(II) is observed. In the pH range 6.5–7.5, Ni**L** is present in the forms $[NiLH]^{3+}$ and $[NiL]^{2+}$, and Co**L** exists primarily as $[CoLH]^{3+}$. All studies below were performed in the pH range 6.5–7.5.

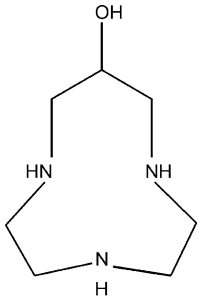

Figure 1. Structure of the ligand **L**.

Table 1. Stability constants for the complex of Ni(II) and Co(II) with ligand **L** (Aqueous 0.1 mol·L^{-1} KNO_3 at 298.0±0.1 K)

Reaction	Logβ
Ni + **L** = Ni**L**[a]	11.09(3)
Ni + **L** + H = Ni**L**H	17.64(4)
Ni + **L** = Ni**L**H$_{-1}$ + H	2.35(5)
Co + **L** = Co**L**	8.93(1)
Co + **L** + H = Co**L**H	16.54(4)
Co + **L** = Co**L**H$_{-1}$ + H	0.34(2)

[a] Charges omitted for clarity.

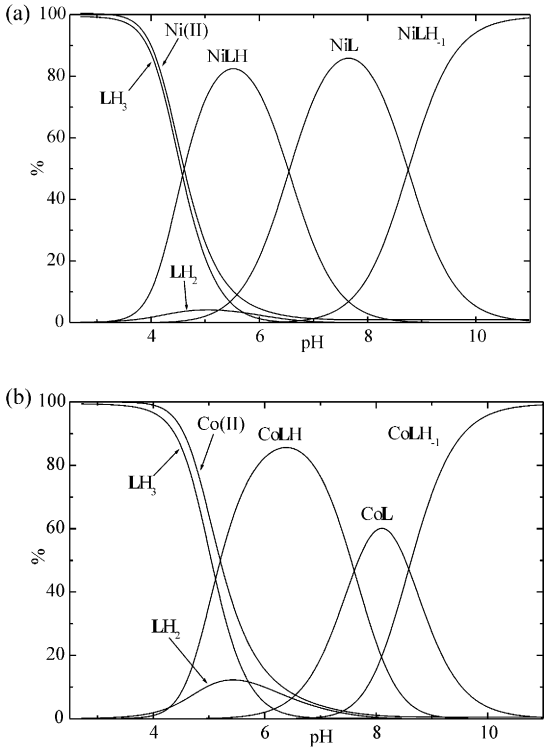

Figure 2. Distribution diagram for the M(II)-**L** system as a function of pH in 0.1 mol·L^{-1} KNO_3 solution at 298.0 K. (a) Ni(II)-**L**; (b) Co(II)-**L**.

Interesting cytotoxic properties in vitro have been detected for investigated Ni(II) and Co(II) complexes. The inhibition percentages were listed in Table 2. It could be observed that Ni(II) or Co(II) exhibited antitumor activity at 10^{-3} mol·L^{-1}; but when Ni(II) or Co(II) was diluted to 10^{-4} mol·L^{-1}, the percentage inhibition decreased sharply. On the contrary, Ni**L** and Co**L** complexes still kept their antitumor activity even at 10^{-6} mol·L^{-1}. These data showed that Cu(II) complex was effective inhibitor of BEL-7402 cell growth, while Ni(II) complex was effective inhibitor of HXO-RB44 cell growth.

Having found that these complexes exhibited antitumor activity, we wanted to determine the possible mechanisms for this behavior. In a study of apoptosis, nuclei of [Ni**L**]-stimulated BEL-7402 cells clearly exhibited condensation and break down into chromatin clumps typical of apoptosis after stained with Hoechst 33258 (Fig. 3);[14] dose response analysis of the induction of apoptosis in BEL-7402 cells by Ni**L** complex indicated optimal induction of apoptosis was at approximately 300 to 400 μmol·L^{-1} (Fig. 4a). In a study of the cell cycle, Ni**L** complex was found to perturb BEL-7402 cells cycle (Fig. 4b). While Co**L** complex did not exhibit introduction effects to BEL-7402 cells apoptosis; and could not perturb BEL-7402 cells cycle.

Table 2. The percentage of growth inhibition data

Compd (mol·L^{-1})	Cell lines					
	BEL-7402			HXO-RB44		
	10^{-3}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-6}
L[a]	34	16	*	3	3	1
Cu(II)[a]	81	3	2	5	3	0
Cu**L**[a]	94	47	11	64	13	2
Ni(II)	89	3	1	6	2	1
Ni**L**	97	23	4	55	32	18
Co(II)	79	5	0	7	3	3
Co**L**	92	29	11	33	19	17

The cells were treated with Ni(II), Co(II), Ni**L** or Cu**L** complex for 48 h and incubated with 5g·L^{-1} MTT for 4 h. The amount of MTT formazan produced was determined by measuring absorbance at 490 nm.[13] All data are represented as the means±S.D. values obtained from five separate cultures.
[a] From ref 10.

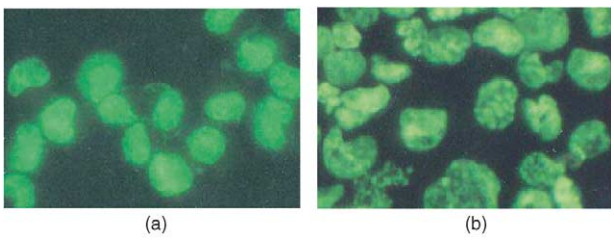

Figure 3. Hoechst 33258 staining of BEL-7402 cells (×400). (a) Normal cells after 48 h of cultivation; (b) cells treated with 3.60×10^{-4} mol·L^{-1} Ni**L** complex after 48 h of cultivation, cells undergoing apoptosis. The cells were stained with 10 mg·L^{-1} Hoechst 33258 fluorescence dye.

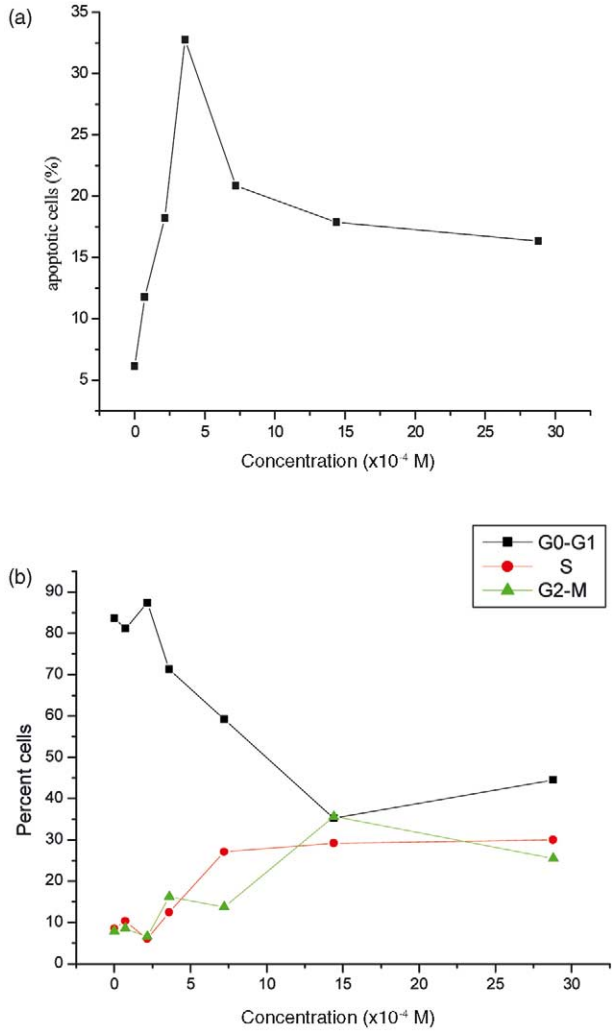

Figure 4. (a) Dose–response curve on [Ni**L**]-induced apoptosis; (b) The perturbation of Ni**L** complex to cell cycle. BEL-7402 cells were treated with 0.721, 2.16, 3.60, 7.21, 14.4, 28.8×10^{-4} mol·L^{-1} Ni**L** complex for 48 h. Cells were then fixed with 70% ethanol and stained with 0.1 g·L^{-1} propidium iodide solution. Cell suspensions were then incubated at 37 °C for 30 min, and stained nuclei were analyzed with a Becton Dickinson FACSort.[16]

The cytotoxic properties of the free ligand **L** were also tested. We found that the ligand **L** is virtually devoid of any intrinsic cytotoxicity (see Table 2). These results strongly support the view that cytotoxicity, for M**L** complexes, can be quite safely ascribed to the presence of the metal center. But the effect of M**L** complexes on apoptosis are markedly different: Ni(II) and Cu(II) complexes could induce apoptosis; while Co(II) complexes could not do this work. Feeblish stabilization of the Co(II) center may result into loss of biological activity, and the apoptosis induced by Cu**L** complex in BEL-7402 cells stained with propidium iodide (PI) was not observed by flow-cytometric analysis,[10] because only apoptotic cells in G_0/G_1 phase could be detected with this method.[15] So mechanisms of apoptosis are also different in tumor cells treated with Ni**L** and Cu**L** complex respectively.

It is commonly believed that the biological activity of anticancer metal complexes is strictly connected to their ability to bind DNA, damage its structure and impair its function.[17] Impairment of DNA function results in inhibition of replication and transcription process and, eventually, if the DNA lesions are not rapidly and properly repaired, in cell death. Macrocyclic nickel and cobalt complexes are attractive compounds because they have the potential to cleave DNA.[18] To investigate whether the Ni**L** or Co**L** complex has direct interaction ability to DNA, we treated it with pBR 322 DNA respectively. In preliminary tests, Ni**L** and Co**L** complexes both showed effective direct interaction on plasmid pBR 322 DNA. With the increase of Ni**L** complex concentration, the supercoiled DNA decreased and converted to nicked and linear DNA at the same time (Fig. 5a); while Co**L** complex only could cleave supercoiled DNA to nicked DNA (Fig. 5b). No significant cleavage was found under comparable conditions by the metal ions alone, in the absence of ligand **L** (Fig. 5c).

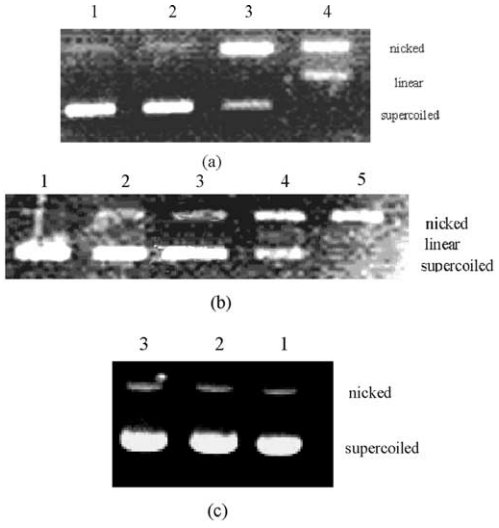

Figure 5. Electrophoresis gel demonstrating cleavage of double stranded DNA (pBR322; concentration 6.1 μmol·L^{-1}) after 2 h at 37 °C and pH 6.5. (a) Lane 1: DNA alone; Lane 2: 50 μmol·L^{-1} Ni(II) complex + DNA; Lane 3: 100 μmol·L^{-1} Ni(II) complex + DNA; Lane 4: 500 μmol·L^{-1} Ni(II) complex + DNA; (b) Lane 1: DNA alone; Lane 2: 10 μmol·L^{-1} Co(II) complex + DNA; Lane 3: 50 μmol·L^{-1} Co(II) complex + DNA; Lane 4: 100 μmol·L^{-1} Co(II) complex + DNA; Lane 5: 500 μmol·L^{-1} Co(II) complex + DNA; (c) Lane 1: DNA alone; Lane 2: 1 mmol·L^{-1} Co(II) ion + DNA; Lane 3: 1 mmol·L^{-1} Ni(II) ion + DNA.

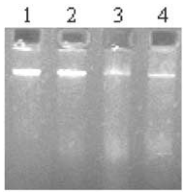

Figure 6. BEL-7402 cells were incubated with or without 300 μmol·L^{-1} M**L** complexes for 48 h. Cells were lysed and the DNA was prepared and electrophoresed on 2% agarose gels and stained with ethidium bromide for detection of DNA fragmentation. Lane 1: untreated cells; Lane 2: cells treated with Co**L** complex; Lane 3: cells treated with Ni**L** complex; Lane 4: cells treated with Cu**L** complex.

In most but not all cases, for apoptotic cells, a putative endogenous endonuclease is activated which digests the DNA at the nucleosomal linker regions resulting in the generation of 'ladder of DNA', which are nucleotide fragments that are 200-bp multimers.[19] In our case, DNA of BEL-7402 cells treated with M**L** complexes was extracted and analyzed on 2% agarose gel as Treves described,[20] and 'ladder of DNA' was not observed (Fig. 6). But it was obvious that DNA of cells stimulated with Cu**L** or Ni**L** complex was damaged, while little cleavage of DNA of cells stimulated with Co**L** complex was found under comparable conditions.

In conclusion, this work demonstrated the nickle(II) and cobalt(II) complexes formation in solution of the ligand [1,4,7]triazecan-9-ol (**L**). Nuclei of [Ni**L**]-stimulated BEL-7402 cells clearly exhibited condensation and break down into chromatin clumps typical of apoptosis. Also it exhibited perturbation effects to cell cycle, and notably optimal induction of apoptosis was found by Flow-Cytometric analysis. But Co**L** complex did not exhibit introduction effects to BEL-7402 cells apoptosis; and could not perturb cell cycle. It is worth noting that Ni**L** and Cu**L**[10] complexes could cleave supercoiled DNA (pBR 322 DNA) to nicked and linear DNA, and DNA of cells treated with Ni**L** or Cu**L** complex was obviously damaged; while Co**L** complex only could cleave supercoiled DNA (pBR 322 DNA) to nicked DNA, and DNA of cells treated with Co**L** complex had no significant difference with control. These results provided a rationale for the antitumor activity of these metal complexes in relation to DNA. Further work is in progress to elucidate the detailed mechanisms of antitumor activity of these complexes.

Acknowledgements

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