· 研究简报 ·

Synthesis, characterization and cytotoxic activity of a new oxaliplatin analog

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新型奥沙利铂类似物的合成、表征与细胞毒性

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摘要: **目的** 合成并表征了一个新的奥沙利铂类似物,顺式-叶酸根·(1*R*,2*R*-环己二胺)合铂(II)([Pt(DACH)FA]),以期获得抗癌活性优于奥沙利铂的新铂配合物。**方法** 采用MTT法,评价了[Pt(DACH)FA]对A549,BGC-823,HCT-116和COLO320人癌细胞株的体外抗癌活性。**结果** [Pt(DACH)FA]在HCT-116和COLO320细胞株中有活性,IC_{50}值分别为50.1 μmol·L^{-1}和25.0 μmol·L^{-1},而在A549和BGC-823细胞株中则活性很低。**结论** 虽然[Pt(DACH)FA]在HCT-116和COLO320细胞株中有一定的抗癌活性,但是在所评价的细胞株中活性均小于阳性对照药奥沙利铂,说明用叶酸根作为解离基团降低了该铂配合物的细胞毒性。

关键词: 奥沙利铂类似物; 表征; 细胞毒性

Introduction

In many countries, colorectal cancer is the leading causes of cancer-related morbidity and mortality, with only 60% of patients alive at 5th year[1,2]. Platinum-based antitumor drugs, as important chemotherapy agents, have been used for the treatment of metastatic disease. However, it has been demonstrated that both cisplatin and carboplatin have no efficacy in advanced colon cancer[3,4]. Oxaliplatin [(*trans*-1*R*, 2*R*-diaminocyclohexane) oxalatoplatinum (II)] is a novel platinum-based antitumor drug developed by Debiopharm (Switzerland) and has been approved for the treatment of colorectal cancer. (Eloxatin^R is commercialized by Sanofi-Aventis in Europe, United States and China)

It is proved now that platinum anticancer drugs *cis*-[A_2PtX_2], such as cisplatin and carboplatin and oxaliplatin, undergo a dissociation reaction after entering tumor cell, forming an active group *cis*-A_2Pt^{2+} and a leaving group X^{2-} or $2X^-$. Attracted by the electrostatic force, the active group moved to the cell nucleus and then binds chemically to DNA. The binding will distort the basic structure and function of DNA, leading to inhibition of DNA replication and then to death of the tumor cell[4]. The drug is believed to be more effective if its leaving group has anticancer activity, for a synergistic effect may be achieved between its two groups.

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A synergistic anticancer effect was clinically observed in the combination use of oxaliplatin with 5FU and folinic acid[3-5]. Based on this effect and the anticancer mechanism of platinum-based drugs, we have prepared a novel oxaliplatin analog through the combination of the active group of oxaliplatin with folic acid, which was potentially improved efficacy and/or toleration.

Results

1 Characterization of oxaliplatin analog

A peak was detected at m/z = 749 in mass spectrum of the oxaliplatin analog which corresponded to the molecular ions although it was very weak. The molecular weight of the analog was 748. The IR spectrum of sodium folate showed three specific C = O absorption bands at 1 690 cm^{-1}, 1 608 cm^{-1} and 1 570 cm^{-1} separately (Table 1). However, we found from the IR spectrum of the analog that the carbonyl on pteridine ring of free folate disappeared after coordination with platinum.

Several groups of peaks appeared in ^1H NMR of the analog (Table 2). These peaks corresponded respectively to the protons of the pteridine ring, benzene ring and the cyclohexane ring. Compared ^{13}C NMR of the analog with free folate, it could be easily seen that the chemical shift (δ 164) of C = O on the pteridine ring of free folate moved to δ 169 after coordination with $DACHPt^{2+}$, while the chemical shifts of other carbonyls remained unchanged. The absorption band at 410 nm was detected in the region of visible-lights. The band was assigned to $d_{xy} \rightarrow d_{x2-y2}$ electron excitation in ligand field of the analog molecule. Based on these structural data, this new analog of oxaliplatin is suggested to have the chemical structure as shows in Figure 1.

2 *In vitro* cytotoxic activity results

The cytotoxic activity was observed with 0.2% $NaHCO_3$ aqueous solution as a solvent. IC_{50} of oxaliplatin and [Pt(DACH)FA] against four tumor cells are listed in (Table 3). It can be seen that oxaliplatin was active on HCT-116, COLO320 cell lines. However, only moderate activity of [Pt(DACH)FA] was found on HCT-116 and COLO-320 cell lines.

Table 2 ^1H NMR and ^{13}C NMR data of analog

Sample	^1H NMR (D_2O, δ)	^{13}C NMR (D_2O, δ)
Analog	8.7(s,1H,C-H,H_a),	169(C_i, C = O)
	7.7(d,1H,J = 7.3 Hz,C-H,H_b)	182(C_m, C = O)
	6.9(d,1H,J = 7.2 Hz,C-H,H_c)	172(C_l, C = O)
	4.4(s,1H,N-CH-COO,H_d)	178(C_n, C = O)
	4.2(s,2H,C-CH_2-NH,H_e)	
	3.7(m,2H,C-CH-NH_2,H_h)	
	2.2 - 2.4(m,4H,-CH_2-,$H_{f,g}$)	
	1.2 - 1.6(m,8H,-CH_2-,$H_{j,k}$)	

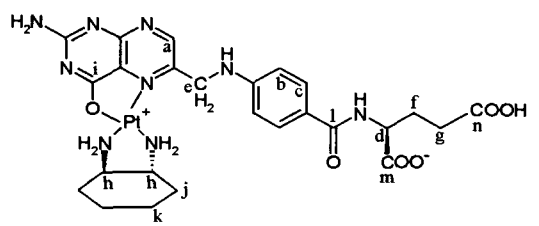

Figure 1 The molecular structure of [Pt(DACH)FA]

Table 3 *In vitro* cytotoxic activity IC_{50} of [Pt(DACH)FA]/μmol · L^{-1}

Cell lines	IC_{50}/μmol · L^{-1}			
	A549	BGC-823	HCT-116	COLO320
Oxaliplatin	188.8	39.2	0.2	11.8
[Pt(DACH)FA]	>133.6	>133.6	50.1	25.0

Discussions

The combination of the active group of oxaliplatin with folic acid allowed us to obtain a new platinum compound. According to the above analyses, the chemical structure of the platinum complex should be consistent with the deduction.

Although [Pt(DACH)FA] exhibits some cytotoxic activity *in vitro*, it is obviously less effective than oxaliplatin. This means that the chemical substi-

Table 1 IR and elemental analysis data of analog and sodium folate

Sample	Mol. Formula	IR/cm^{-1}	Elemental analysis/% Calc(Found)			
			C	N	H	Pt
Sodium folate	$C_{19}H_{17}N_7O_6Na_2$	1 690($C_{m,n}$ C = O)	47.0	20.2	3.5	
	[485.4]	1 608(C_l, C = O)	(47.1)	(20.4)	(3.7)	
		1 570(C_i, C = O)				
Analog	$C_{25}H_{31}N_9O_6Pt$	1 706($C_{m,n}$ C = O)	40.1	4.1	16.8	25.7
	[748.4]	1 608(C_l, C = O)	(40.3)	(4.2)	(17.1)	(26.0)

tution of oxalate with folate as the leaving group reduces cytotoxic activity of oxaliplatin. The reason for the decrease in activity is not yet understood but may be related to the difficulty in cellular uptake due to the large size of [Pt(DACH)FA] molecule and/or to its stability[6]. The great stability of [Pt(DACH)FA] leads to a difficulty in releasing the active group of oxaliplatin. Although [Pt(DACH)FA] does not meet the traditional structure-activity relationships of Pt-based antitumor drugs, it did show some cytotoxic activity *in vitro*, which may provide a new approach to Pt-based drug design.

Experimental

All chemicals and solvents used were analytically pure. IR spectrum of the platinum complex was recorded in 4 000 – 400 cm^{-1} regions on a Perkin-Elmer 880 spectrometer with KBr pellets and IR spectrum of sodium folate was measured at the same time as a control. The mass spectrum of the complex was measured on a VG-Auto300 spectrometer in the FAB^+ mode using glycerine as matrix. ^1H NMR and ^{13}C NMR were performed on Brucker AM-400 in D_2O solution of 2% $NaHCO_3$. The electronic spectrum of the complex in aqueous solution of 2% $NaHCO_3$ was measured on Shimadzu UV-2201 spectrometer.

1 Preparation of oxaliplatin analog

Cis-[Pt(DACH)I_2] was used as a starting material and hydrolyzed with $AgNO_3$ in aqueous solution into *cis*-[Pt(DACH)$(H_2O)_2$]$(NO_3)_2$ at 35 ℃. Sodium folate was prepared by neutralization of folic acid with sodium hydroxide. Upon the addition of sodium folate into *cis*-[Pt(DACH)$(H_2O)_2$]$(NO_3)_2$, a brown-orange product precipitated from the mixture solution. After being collected by filtration, it was washed with water and ethanol, and then dried at 60 ℃. The product was purified in an aqueous solution of 3% $NaHCO_3$. The oxaliplatin analog was insoluble in water, ethanol and acetone, but slightly soluble in weak acid or alkaline solution, such as dilute trifluoroacetic acid and dilute $NaHCO_3$.

2 *In vitro* antitumor assays

2.1 Cell culture A549 (human lung cancer cell lines), BGC-823 (human stomach cancer cell lines), HCT-116 and COLO320 (human colorectum cancer cell lines) were cultured in the RPMI-1640 medium containing 10% fetal bovine serum, penicillin 1 × 10 $U \cdot L^{-1}$ and streptomycin 0.1 $g \cdot L^{-1}$, at 37 ℃ in a humidified atmosphere of 5% CO_2. The cells grew as monolayers in tissue culture flasks and were subcultured approximately once every 3 – 5 days by trypsinization.

2.2 MTT assay The cells at the exponential growth stage were adjusted to adequate density. Then, the living cells were implanted in 96 well culture dishes at density of 90 μL per well. After the cells adhered to the walls, 10 μL medium containing five different concentrations of the antitumor drug solution were added in treble for each concentration. The culture medium was the negative control and oxaliplatin was the positive control. After being incubated at 37 ℃ in a humidified atmosphere of 5% CO_2 for 72 h, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, concentration of 5 $g \cdot L^{-1}$) was added (20 μL per well). Four hours later, the supernatant was discarded and sodium dodecylsulfate (SDS, 10%)-isobutyl alcohol (5%)-hydrochloric acid (0.012 mol $\cdot L^{-1}$) (100 μL per well) was added to dissolve the formazan. The optical density (OD) was measured on an EL340 BIOTECK reader at 570 nm. The grow inhibitory rate (%) (IR) was calculated according to the following formula: IR = (1 – mean OD of cells with test compound/mean OD of negative control culture medium cells) × 100%. IC_{50} was calculated according to LOGIT method.

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