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Primary screening for antitumor activity of Tetracycline-platinum (II) complex using Ultraviolet spectroscopy

التحري الاولي عن الفعالية المضادة للاورام للمعقد البلاتين الثنائي مع التتراسايكلين باستخدام طيف الاشعة فوق البنفسجية

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Abstract:

In order to further clarify the molecular basis for the mechanism of action of the antitumor Pt(II) complexes, this work represent a study of the interaction of Pt(II) complex with calf thymus DNA and Human Serum Albumin (HAS) using UV-spectroscopy in vitro, this technique was used in order to gain quantitative information regarding the relative binding affinity of the platinum compounds for calf thymus DNA versus HAS.

Spectra of solution of Pt(II) complex (0.07 mM) at pH_7 was recorded at regular intervals in the absence and presence of calf thymus DNA (0.05mM) and HAS (0.16µM). The present study reveals that the UV spectrum of Cis-dicloro(tetracycline) platinum(II) complex [(Tet.)Pt Cl_2] at pH_7 pale yellow solution shows a maximum absorbance at 272nm and a shoulder peak at 364nm after 0.25h and 1 hr . These peaks are rapidly diminished with time due to rapid hydrolysis of the nephthacene rings to give a precipitate and loss of the pale yellow color of the solution after 24hrs. In contrast, the absorbance of platinum complex in the presence of calf thymus DNA and HAS is significantly altered compared to the control experiment. The shift in the maximum absorbance to 291nm and 290 (for complex with DNA and HAS respectively) from 272nm implies that the tetracycline ligand is in a different environment, consistent with formation of platinum – DNA and platinum-HAS complex. No precipitation occurred in solution, of platinum complex in the presence of calf thymus DNA and HSA at pH_7 over 24h.

Key words: Antitumor activity. UV-visible spectroscopy, platinum (II) complex, in vitro

الخلاصة:

تضمنت الدراسة الحالية التحري الاولي عن الفعالية المضادة للاورام خارج الجسم الحي لمعقد البلاتين الثنائي مع التتراسايكلين بمفاعلته مع جزيئة الحامض النووي الرايبوزي DNA والبومين المصل البشري HSA باستخدام تقنية طيف الاشعة فوق البنفسجية UV—spectroscopy.

تم قياس طيف الشعة فوق البنفسجية لمحلول المعقد البلاتيني (PH 7 -0.07mM) بوجود الجزيئات الحيوية DNA (0.05mM) و HSA (0.16 mM) وعدم وجودها باوقات متتالية (0.25 و 1 و 24 ساعة) فضلا عن قياس طيف الاشعة فوق البنفسجية للجزيئات الحيوية القياسية. اعطى طيف المعقد (محلول اصفر شاحب) اعلى قمة امتصاص عند الطول الموجي 272 نانومتر وكتف عند الطول الموجي 364 نانومتر بعد مرور 0.25 , 1 ساعة , في حين اضمحلت القمم وفقد المحلول لونه وظهر راسب يشير الى تفككك المعقد بعد مرور 24 ساعة . من جهة اخرى , يعطي تفاعل المعقد المباشر مع احدى الجزيئات الحيوية DNA او HSA تغيرا ملحوظا في مواقع القمم بحصول ازاحة في قيم الامتصاص من 272 نانومتر (في المعقد بدون DNA و HSA) الى 290 و 291 نانومتر (في المعقد مع DNA و HSA على التوالي) مما يؤكد حصول تفاعل وارتباط بين المعقد البلاتيني والجزيئات الحيوية بحيث لا يحدث تفكك او ترسيب للمركب الناتج بعد مرور 24 ساعة من التفاعل.

الكلمات المفتاحية: الفعالية المضادة للاورام , طيف الاشعة فوق البنفسجية, معقدات البلاتين الثنائي , خارج الجسم الحي

Introduction

Anticancer drugs can be classified into the categories, intercalators, minor groove binders, alkylating agents and inorganic coordinative complexes depending on their mode of interaction with DNA. Cisplatin is one of the most potent and widely used anticancer drugs known,its formal name *cis*-diamminedichloroplatinum (II), or *cis*- [Pt $(NH_3)_2$ Cl_2] abbreviated as *cis*-DDP and CDDP [1,2,3]. The discovery of cisplatin as anticancer agent led to an intense interest in metal complexes as anticancer drugs metals are electron deficient as opposed to biomolecules such as DNA proteins that have many electron rich binding sites leading to strong interactions between them [4,5] . The reaction of cisplatin with DNA leads to binding of Pt(II) to guanine (s) at N_1- sites giving several types of DNA adducts: mono adducts, interstrand crosslinks, intrastrand crosslinks and DNA protein cross links. As a result of the formation of DNA intrastrand crosslinks a specific distortion of DNA occurs , which seems to be linked with the cytotoxicity and antitumor activity of cisplatin [6,7]. In contrast, Human serum albumin HSA, a major metal transport protein (52%) of total weight of human plasma proteins. HAS is known to bind and transport metals such as copper (II) , nickel (II), calcium(II) and zinc(II) in the blood. In general, HAS preferentially binds of metals through soft-nitrogen donors in a square-planar geometry [8,9].

Cytotoxicity is the cell-killing property of a chemical compounds, terms such as cell survival, cell killing, integrity, and cytotoxicity are often arbitrarily employed [10]. There are now a number of in vitro cytotoxicity tests capable of detecting agents which interact with DNA, modified DNA, or both such as. Screening for antitumor agents requires assays that are rapid, inexpensive, and amendable to large –scale screening. *In vivo* tumor test systems meet none of these criteria, and mammalian cell culture assays are also slow and expensive. Therefore, *in vitro* microbiological assays systems are frequently used for the initial screening for antitumor compounds [11].

Ultra-Violet spectroscopy is a technique commonly used to study the interaction of a substance (e.g. a drug) with biomolecules including DNA and proteins [12]. A UV-Vis spectrophotometer measures the amount of light absorbed at each wavelength of the UV and visible regions of the electromagnetic spectrum. The wavelength of absorption is usually reported as λmax which represents the wavelength at the highest point of the curve. The structural unit associated with an electronic transition in the UV-Vis spectroscopy is called a chromophore [13,14].

The advantage of the technique is that it can be carried out at low sample concentrations with solutions that approximate physiological conditions, provided the sample contains a suitable chromophore that absorbs light at a wave length that can be monitored [15] .Typically, an interaction on association is apparent when there is a shift in the maximum absorbance wave length or when there is a change in the absorbance at a selected wave length. As each spectrum can be recorded in a relatively short period of time, a range of sample concentrations can be screened [16,17].

Aim of the study

This study focused on the DNA damaging properties of Cis-dicloro(tetracycline) platinum(II) complex *cis*-[(Tet.)Pt Cl_2], a novel analogue of cisplatin with tetracycline antibiotic used as non-leaving ligand using UV- spectroscopy to monitor the interaction of complex with calf thymus DNA and Human serum albumin HSA depending on the alteration in the secondary structure of nucleic acid DNA and protein when they interact with metal complexes.

Materials and Methods

(1)Synthesis of *Cis*-dichloroplatinum (II) complex:

Cis-[Pt(Tet.) Cl_2] complex was synthesized as described earlier [18]. A water solution of potassium terachloroplatinate K_2Ptcl_4 (0.418g, 1mM, BDH, England) was added to tetracycline antibiotic bidentate ligand (0.474g, 1mM) in ethanol (80ml).The reaction mixture was stirred for 6 hrs at room temperature away from light. The precipitate of was filtered, washed with ethanol and dried overnight at room temperature.

(2)Preparation of platinum(II) compound concentration:

Stock solution of [(Tet.)$Pt(Cl)_2$] complex was prepared by dissolving (0.7gm, 2.8 µmole) in 1% dimethylsulfoxide DMSO (ICN-USA) immediately before use. The PH of solution was adjusted with sodium hydroxide (1% w/v) as required.

(3) Preparation of DNA and HSA solutions:

Calf thymus DNA was purchased from Sigma Company and used without further purification. A stock solution was prepared by dissolving DNA (1.8mg) in water (100ml) by stirring at room temperature for 1hr.The purity of the DNA solution was verified from the ratio (A260nm/A280nm) which was close to 1.85.

Human serum albumin (HSA, MW 68500) was purchased from Sigma Company and used without further purification. A stock solution was prepared by dissolving HSA (0.9mg, 0.013 µmol)in water (20 ml) by stirring for 1hr.

(4)Ultra-violet studies of platinum complex, DNA and HSA :

The UV spectra of complex (0.07mM) in water at pH (7-7.4) was recorded at 0.25 hr, 1hr and 24hrs at 25^{o}C. The UV spectra of HSA (0.16µM) and calf thymus DNA (0.05mM) in water were recorded at 1hr and 24hrs at 37 ^{o}C so as to provide blank experiments. Aliquots of DNA, HAS and complex solutions were taken at different times of incubation.

(5)UV- binding studies of DNA and HAS with complex :

The UV binding experiment with calf thymus DNA involved recording the UV spectra over 24hr of solution of calf thymus DNA (0.05 mM) with complex (0.07 mM) at PH (7.0-7.5, incubated at 37^{o}C for 0.25 hr , 1hr and 24 hrs. Aliquot of DNA-complex solution was taken at different times of incubation. The UV absorbance of blank experiments with calf thymus DNA under identical condition was subtracted from the UV absorbance recorded in the presence of the complex.

The UV binding experiment with HSA involved recording the UV spectra over 24hr of solution of HSA (0.16µM) with complex (0.07 mM) at PH (7.0-7.5), incubated at 37 ^{o}C for 0.25hr ,1hr and 24 hrs. Aliquot of HSA-complex solution was taken at different times of incubation. The UV absorbance of blank experiment with HAS under identical condition was subtracted from the UV absorbance recorded in the presence of the complex.

Results

(1)Synthesis of *Cis*-dichloroplatinum (II) complex:

The direct reaction of one mole of antibiotics (An.) with one mole of K_2PtCl_4 gives [(An.) Pt Cl_2] complex . The complex was soluble in dimethylsulfoxide (DMSO), dimethylformamide (DMF), and chloroform. The prepared complex decomposed in the temperature range (222-225) ^{o}C. Physical properties of prepared complex was shown in (Table 1. Fig 1).

(2)UV- binding studies: (Figure 2)

In order to determine whether *Cis*-dichliro(tetracycline)platinum(II) complex show any interaction or association with DNA and HSA, the UV spectra of aqueous solution of Pt(II) complex (0.07 mM) at PH7 was recorded at regular intervals in the absence and presence of calf thymus DNA (0.05mM) and HAS (0.16µM).

On the other hand, the absorbance of calf thymus DNA (0.05mM)and HSA (0.16µM) was independently measured over the same time to represent blank experiments and frame and subtracted from the UV absorbance measured in presence of complex. Both Standard DNA and HAS shown λ max at 261 nm.

Discussion

In a study performed by [18], tetracycline antibiotic ligand was used as non- leaving group in the synthesis of platinum (II) complex. Antibiotic such as tetracycline is bioactive molecule with reactive properties, can produce numerous derivatives having a wide range of uses, thus increased the chance of their coordination to the platinum metal center.

The UV spectrum of [(Tet.)Pt Cl_2] in water at PH7 pale yellow solution shows a maximum absorbance at 272nm and a shoulder peak at 364nm after 0.25h . These peaks are rapidly diminished with time due to rapid hydrolysis of the nephthacene rings to give a precipitate and loss of the pale yellow color of the solution after 24hrs.

In contrast, the absorbance of platinum complex in the presence of calf thymus DNA is significantly altered compared to the control experiment. Firstly, the shift in the maximum absorbance to 291nm from 272nm implies that the tetracycline ligand is in a different environment, consistent with formation of platinum – DNA complex, the UV absorbance is also increased initially by approximately 20%. Secondly, no precipitation occurred in solution, of platinum complex in the presence of calf thymus DNA at PH7 over 24h.The observation indicate that nephthacine hydrolysis processes are altered in the presence of calf thymus DNA at PH7 and supported the UV data, which indicate that there is an interaction or association of complex with calf thymus DNA.

Similar experiment was carried out for the same platinum complex with HSA. The absorbance at 272 nm increases significantly to 290nm with time. As in the case of complex in the presence of HSA, the absorbance of complex is significantly altered compared to the control experiment. The altered absorbance is consistent with an interaction between complex and calf thymus DNA and HSA.

The use of amines more compatible to the human system has been another important area, for this purpose, naturally occurring substances like antibiotics amino acids, peptides and glucosamines whose uptake is increased in malignant cells have been used as non- leaving ligands in some platinum complexes [19]. The replacement of ammonia groups in the cisplatin molecule by bulky ligand (such as tetracycline) may influence the rate of the activity of resultant complex. Reedijk show that the Pt(II) complexes with bulky ligands may increase the reaction of these complex with DNA [6]. As well as, the reactive complexes are able to form both intrastrand and interstrand DNA adducts. Metal ions play a key role in the actions of synthetic and normal metalloantibiotics, and are involved in specific interactions of these antibiotics with proteins, membranes, nucleic acids, and other biomolecules. Metal centers being positively charged, are favoured to bind to the negatively charged biomolecules [20,12].

It is now widely accepted that the anticancer properties of cisplatin are the result of specific interactions of the cis-$[Pt(NH2)_2]^{+2}$ fragment with DNA. The possible binding sites on DNA include the N_7 atom of guanine and adenine, the N_1 atom of adenine and atom of cytosine .out of these four possible sites, cisplatin shows a strong preference for the N_7 site of guanine .The preference seems to be related to the stronger basicity of guanine-N_7 atom and hydrogen bonding .The physical nature of DNA is important to consider due to its fundamental importance in metal DNA interaction [21].

The low PKa value of the phosphate groups makes the DNA highly charged molecule in a large PH range. As a result, the charge density is high, and the DNA attracts oppositely charged ions and repels negatively charged ions. In this respect, the nucleobases acts as ligands and thus coordinates to the metal of an already positively charged species [22,23].

In conclusion, the results of this study, confirmed the ultraviolet spectroscopy method to be a simple, cheap, sensitive and accurate biochemical assay suitable for screening programs designed to detect agents that damage DNA and that are potential interest in carcinogenesis and cancer chemotherapy.

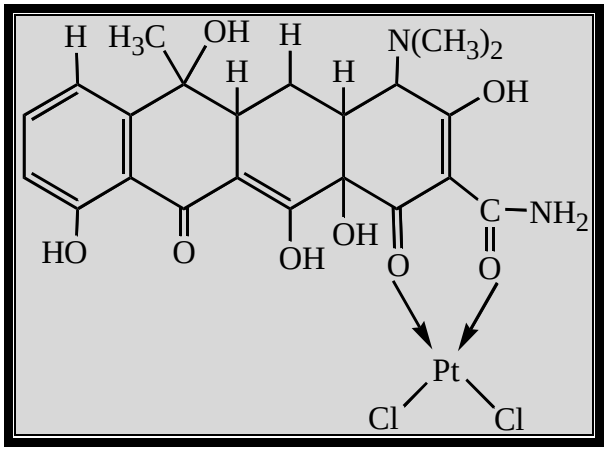


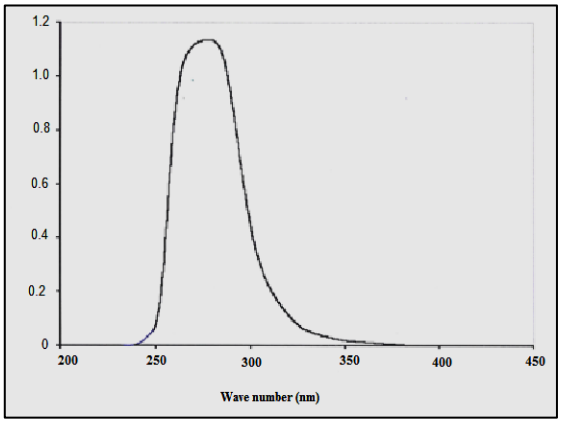
Figure (1): Tetracyclino- dichloroplatinum(II) complex.

Table (1): Some physical properties of the prepared complexes

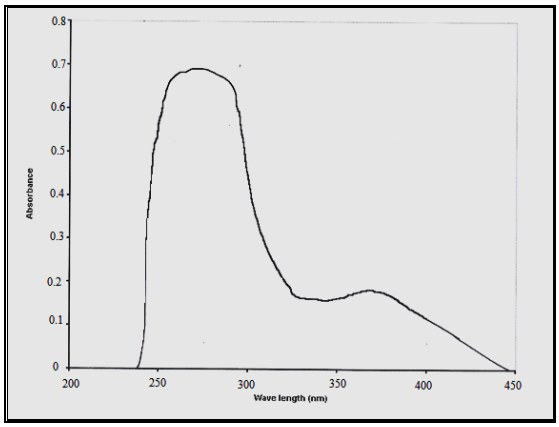
Compounds	Physical properties	
	Color	Melting point(dec)^{o}C
Tetracycline standard	Yellow	(170-175)
[(Tet.) Pt (Cl_2)] complex	yellow-Beige	(222-225)

Table (2): UV-Visible spectra of tetracycline- platinum (II) complex in the presence and absence of DNA and HAS.

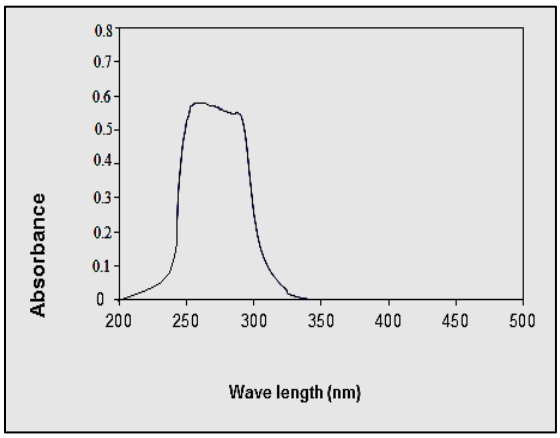
Compounds		λ max (nm)	Color of solution after 0.25 and 1hr	Color of solution after 24hrs	Chemical shift %
1-	Tetracycline standard	278	yellow	Dark brown	-----
2-	Calf thymus DNA standard	261	white	------	-----
3-	Human serum albumin (HAS) standard	261	Pale yellow	------	-----
4-	[(Tet.)Pt Cl_2] complex	272, 364	yellow	Clear solution with yellow precipitate	-----
5-	[(Tet.)Pt Cl_2] complex +DNA	291, 369	Pale yellow	Pale yellow	20 %
6-	[(Tet.)Pt Cl_2]Complex +HSA	290, 366	Pale yellow	Pale yellow	10 %



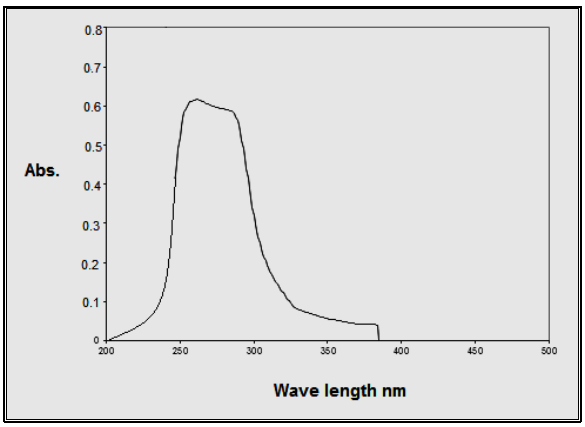
Tetracycline standard



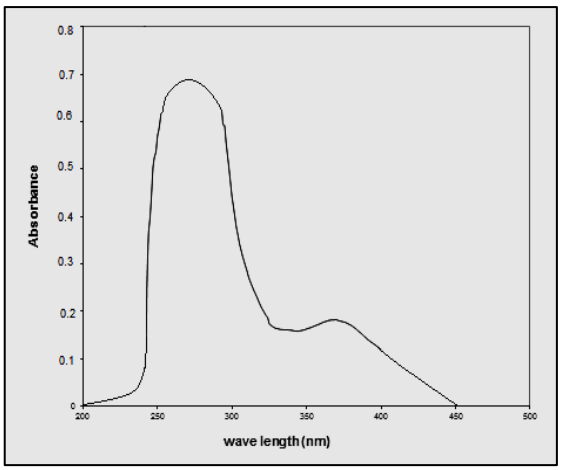
[(Tet.)Pt(Cl)$_2$] complex



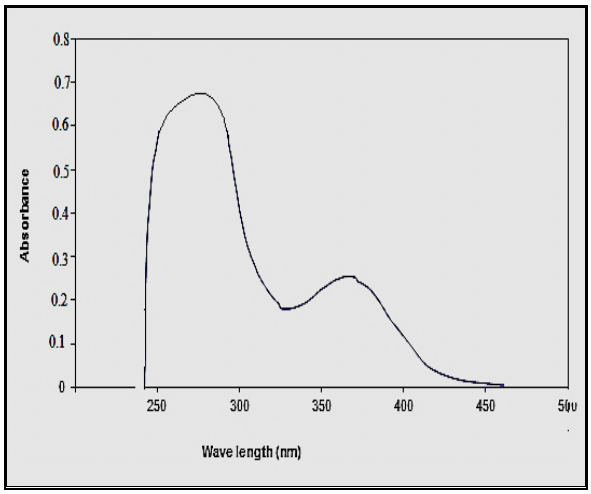
DNA standard



HAS standard



[(Tet.)Pt(Cl)$_2$] complex+DNA



[(Tet.)Pt(Cl)$_2$] complex+HSA

Figure (2): UV-Visible spectroscopy of test compounds.

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