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Mediterranean Journal of Chemistry

Mediterranean Journal of Chemistry **2018**, 7(1), 28-38

Relative cytotoxicity of complexes of platinum(II) and palladium(II) against pure cell culture *Paramecium caudatum* and human cell lines A431 and HaCaT

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Abstract: The results of cytotoxicity cis-diamine mono- and binuclear complexes of platinum(II) and palladium(II) are presented. The cytotoxicity was investigated by the method of biotesting with *Paramecium caudatum* and by MTT-assay with human cells: epidermoid carcinoma A431 and minimal transformed aneuploid keratinocytes HaCaT. Cytotoxicity of complexes towards protists is higher than against human cells, however, comparatively, HaCaT is more sensitive than A431 by the treatment all complexes. It is noted that cytotoxicity of palladium(II) complexes is higher than the analogues with platinum(II).

Keywords: complexes, platinum, palladium, hydrolysis, toxicity, *Paramecium caudatum*, A431, HaCaT, MTT-assay.

Introduction

B. Rosenberg's discovery of cytotoxic properties of cis-$[Pt(NH_3)_2Cl_2]$ (Cisplatin) in 1969 marked an emergence of a new class of antitumoral drugs – coordination compounds of d-elements [1]. From the end of the 1970s there was a wide clinical use of cisplatin and since then it is actively spread in modern courses of a chemotherapy of some oncologic diseases [2-4].

Almost all cisplatin analogues (well-known Carboplatin, Oxaliplatin, etc.) are structurally similar: the central ion of Pt(II) is in square planar shape, formed by cis-amino and acid ligands. Amino- (nitrogen-containing) ligands, except some cases [5], determine the main cis-conformation of complex and due to its trans-affinity [6] labile mono- or bidentate acid ligands, leading to the complex forms that are capable to enter biochemical processes of intercalation and metabolism [5].

In vitro, being dissolved in water, and/or *in vivo*, in the conditions of lowered intracellular concentration of chloride ion, cisplatin (or its analogues) suffers hydrolysis, forming cis-$[Pt(NH_3)_2(H_2O)Cl]^+$ and cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (Fig. 1), interacting with nuclear DNA [4] - with replacement of water molecules to guanine (G). Furthermore, macrochelate cycle usually closes, forming adducts of the type cis-$[Pt(NH_3)_2\{N7,N7\text{-}GG\}]$, where platinum coordinated to two neighbouring guanine bases of one strand of DNA, or cis-$[Pt(NH_3)_2\{N7,N7\text{-}AG\}]$, where coordination goes to the neighbouring nucleotides – adenine (A) and guanine [7]. The formed adducts block the translation and replication processes of DNA, as a result, leading to apoptosis [8-10].

Nevertheless, among a huge number of researchers, that were devoted to metal complexes and their antitumor properties, there are practically no works dedicated to cytotoxicity of the complexes, considering toxicity of hydrolytic and "inactivated" forms which are formed over time in solutions of drugs ("ageing of solutions") and in processes of metabolism (biotransformation). It is known that both in *situ*, and *in vitro* complexes of platinum (II) [4,11] and palladium (II) [12,13] are undergoing hydrolysis. Process starts from increasing lability (because of trans-affinity effect of nitrogen-containing ligands from inner sphere of complex) and substitution water molecules to acid ligands (e.g. – chloride ion) forming cis-$[M(N\cap N)_2(OH_2)_2]^{2+}$ (where N∩N = 1,10-phenanthroline (phen), 2,2`-dipyridyl (dipy), two molecules of NH_3 in cis-form, ethylenediamine (en), etc.) [11,12].

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DOI: http://dx.doi.org/10.13171/mjc71/01803131026-eremin

Received March 13, 2018
Accepted, March 21, 2018
Published April 9, 2018

During the process, a number of protolytic equilibriums are observed, following by deprotonation and oligomerization of generated complexes and mix formation of bi-, tri- or oligonuclear forms of the type (Fig. 1) [14,15]: $[((N\cap N)M(OH_2))_2(\mu\text{-}OH)]^{3+}$, $[M_2(N\cap N)_2(\mu\text{-}OH)_2]^{2+}$, $[M_3(N\cap N)_3(\mu\text{-}OH)_3]^{3+}$, etc. In a physiological range of pH, complex forms of $M(N\cap N)(OH)(OH_2)^+$ and $[M_2(N\cap N)_2(\mu\text{-}OH)_2]^{2+}$ are dominated [16].

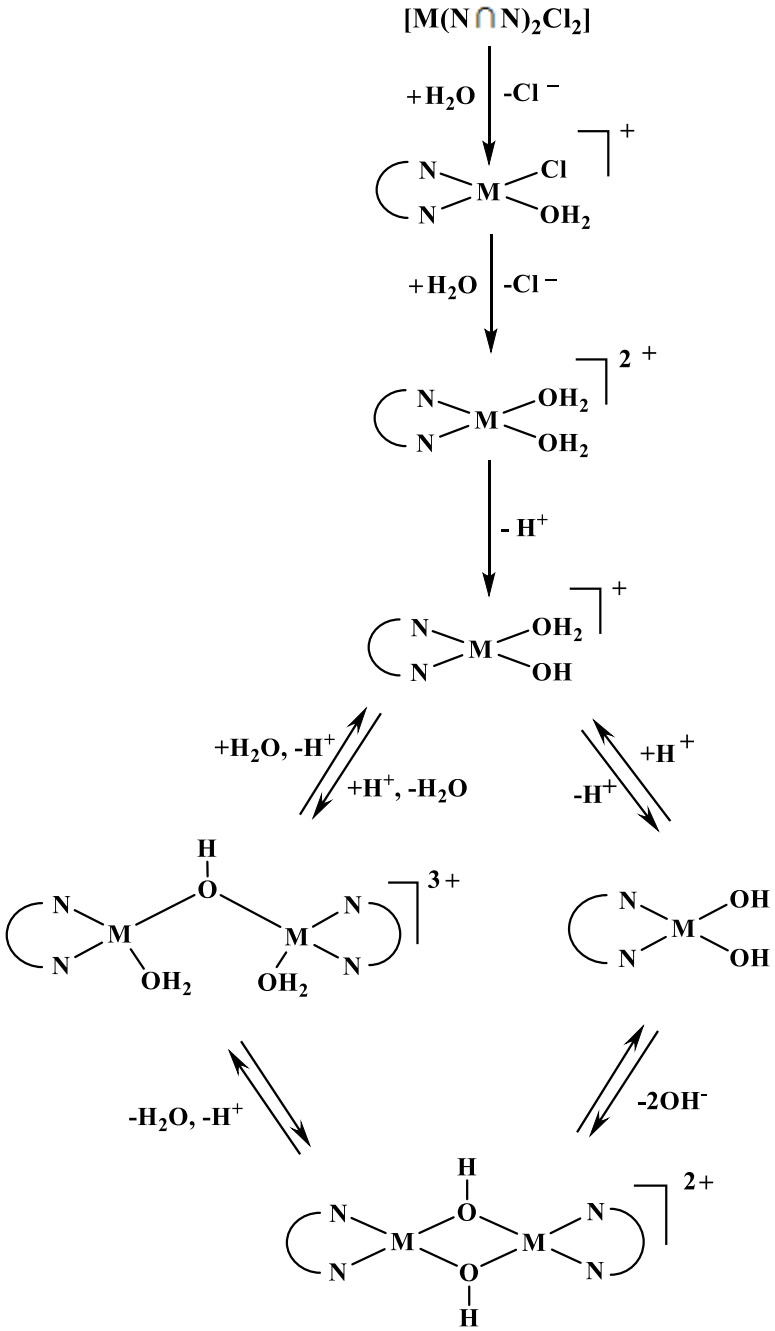


Figure 1. The main pathways of formation hydrolysis forms of complexes cis-$[M(N\cap N)_2Cl_2]$ (M = Pt(II), Pd(II))

Just as the initial complexes cis-$[M(N\cap N)_2Cl_2]$ (M = Pt(II), Pd(II)) and their hydrolysis forms (Fig. 1) are capable as well to react with intracellular sulfur containing molecules, which are the main inactivators of platinum compounds and other heavy metals [17,18] – aminoacids (cysteine (Cys) and methionine (Met)), low-molecular peptides (e.g, glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) or its disulfide forms [19,20] in the sum of components ~90% of a cellular pool of nonprotein sulfur in an organism [21].

In this paper synthesized compounds **II-VIII** represent model complexes of original cisplatin, a product of its hydrolysis and inactivated form with a thiol. Choice of this complexes is caused by their structural similarity with cisplatin and its forms *in vivo*.

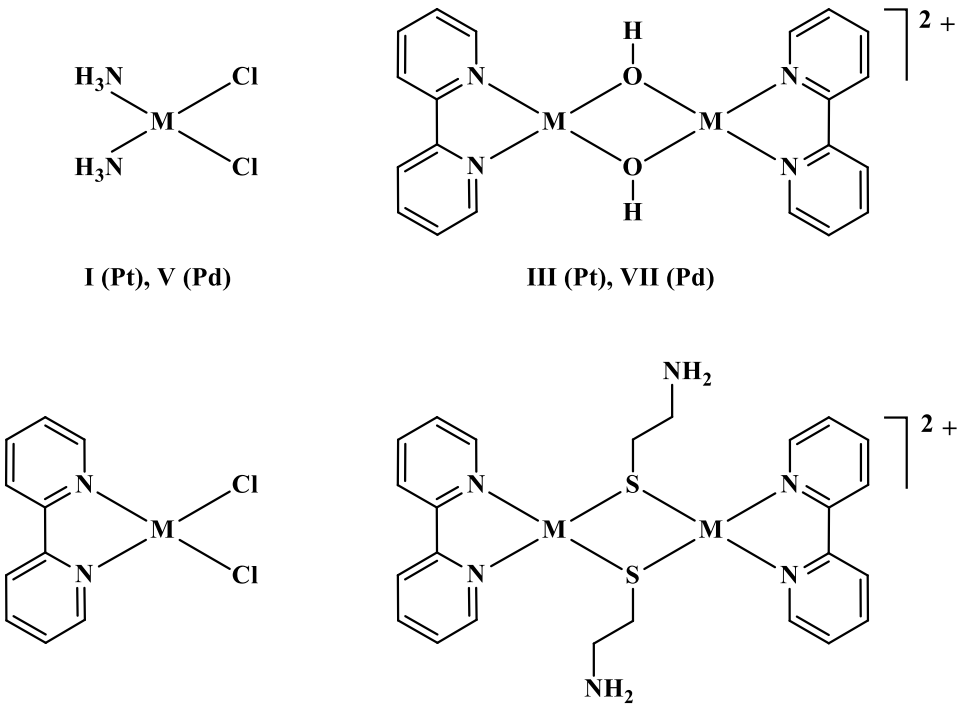


Figure 2. **I-VIII** complexes (M = Pd(II), Pt(II))

A solution of compounds **I-VIII** were tested on monoclonal culture *Paramecium caudatum* by a biotesting method with receiving of death curves and cytotoxicity of these compounds was determined by well-known MTT-test on human normal and cancer cell with the same histological genesis with receiving of LD_{50}.

Materials and methods

Chemicals

2,2`-Dipyridile, 2-aminoethanthiol hydrochloride (aet), 3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich; NaCl, KCl, $CaCl_2 \cdot 2H_2O$, $MgCl_2$, $NaHCO_3$, NH_3 (25% aqueous solution), HNO_3 (65% aqueous solution), HCl (36% aqueous solution), 2-propanol from Acros.

cis-$[Pt(NH_3)_2Cl_2]$ (**I**) was purchased from Sigma Aldrich or was synthesized according to the method given in [22]. To clean the complex, it was recrystallized from 1M hydrochloric acid. The purity of traces of trans-isomers was confirmed according to the method given in [23]. For the **I** it was recieved, %: Pt - 65,1; Cl - 23,1; N - 9,05. And calculated for $PtN_2Cl_2H_6$, % : Pt - 65,05; Cl - 23,63; N - 9,34.

Complex $[Pt(dipy)Cl_2]$ (**II**) was synthesized according to the literature [27]. For the **II** it was retrieved, %: Pt - 42,49; C - 28,10; N - 6.55. And calculated for $PtN_2C_{10}H_8Cl_2$, %: Pt - 46,20; C - 28,45; N - 6,64. IR (KBr): ν_{max}, $см^{-1}$: 430(m), 718(s), 763(s), 1160(m), 1450(s), 1610(m), 2361(w.br), 3084(w.br).

$[Pt_2(\mu\text{-}OH)_2(dipy)_2](NO_3)_2 \cdot 2H_2O$ (**III**) were synthesized according to the literature [24]. For the **III** it was retrieved, % : Pt - 42.89; C - 26,40; N - 9,00. And calculated for $Pt_2C_{20}H_{22}N_6O_{10}$, %: Pt - 43,51; C - 26,79; N - 9,37. IR (KBr): ν_{max}, $см^{-1}$: 520(m), 576(s), 1032(s), 1625(m), 3440(m), 3520(m), 3590(sh).

$[Pt_2(\mu\text{-aet})_2(dipy)_2](NO_3)_4 \cdot 2H_2O$ (**IV**) were synthesized according to the literature [20]. For the **IV** it was retrieved, %: Pt - 34,15; C - 25,26; H - 2.92; N - 12,20; S - 5,61. And calculated for $Pt_2C_{24}H_{34}N_{10}O_{14}S_2$, %: Pt - 34,20; C - 25,27; H - 3,00; N - 12,28; S - 5,62. IR (KBr): ν_{max}, $см^{-1}$: 721(m), 774(m), 1161(m), 1319(v.s), 1370(v.s), 1384(v.s), 1452(m), 1472(m), 1610(s), 3041(m), 3052(m), 3078(m), 3123(m), 3428(w.br), 3500(w.br).

cis-$[Pd(NH_3)_2Cl_2]$ (**V**) was synthesized according to the literature [22]. For the **V** it was retrieved, %: Pd - 50,23; Cl - 33,12; N - 13.13. And calculated for $PdN_2Cl_2H_6$, %: Pd - 50,34; Cl - 33,54; N - 13,25. IR(KBr): ν_{max}, $см^{-1}$: 500(m), 512(w).

$[Pd(dipy)Cl_2]$ (**VI**) were synthesized according to the literature [25]. For the **VI** it was retrieved, %: Pd - 32,00; Cl - 21,05; N - 8,26. And calculated for $PdN_2C_{10}H_8Cl_2$, %: Pd - 31,90; Cl - 21,26; N - 8,39. IR(KBr): ν_{max}, $см^{-1}$: 651(m), 692(s), 761(s), 1097(m), 1161(m), 1435(s), 1481(s), 2361(w.br), 3051(w.br).

$[Pd_2(\mu\text{-}OH)_2(dipy)_2](NO_3)_2 \cdot 2H_2O$ (**VII**) were synthesized according to the literature [26]. For **VII** it was received, %: Pd - 29,91; C - 33,25; N - 11,56. And calculated for $Pd_2C_{20}H_{22}N_6O_{10}$, %: Pd - 29,59; C

- 33,40; N - 11,68. IR(KBr): ν_{max}, $см^{-1}$: 300(w), 520(w), 560(s), 1025(s), 3400(m.br).

$[Pd_2(\mu\text{-S-aet})_2(dipy)_2](NO_3)_4$ (**VIII**) were synthesized according to the literature [27]. For **VIII** it was received, %: Pd - 22,20; C - 29,56; H - 3.42; N - 14,35; S - 6,61. And calculated for $Pd_2C_{24}H_{34}N_{10}O_{14}S_2$, %: Pd - 22,12; C - 29,91; H - 3,56; N - 14,54; S - 6,65. IR(KBr), ν_{max}, $см^{-1}$: 435 (m), 707 (s), 780 (m), 744 (s), 1334 (m), 1425 (s), 1494 (s), 1512 (m), 1582 (m), 3050 (w.br).

Cells

Monoclonal culture *Paramecium caudatum* was obtained from the department of molecular biotechnology of Saint-Petersburg State Institute of Technology. It was cultured in Losin-Losinski's buffer saline (NaCl 0,01%, KCl 0,001%, $CaCl_2$ 0,001%, $MgCl_2$ 0,001%, $NaHCO_3$ 0,002%, Mass.) with *Bacillus subtilis* as a single source of food. Cells were maintained at 25ºC in a humidified atmosphere in the air in dark.

Two human cell lines were used for cytotoxicity determination: A431 (epidermoid carcinoma) and HaCaT (minimal transformed immortalized human keratinocyte). Its obtained from Russian cell culture collection of Institute of Cytology (RAS) and cultured in DMEM medium (Gibco, ThermoFisher) with high glucose and 4 mM L-glutamine, supplemented with 10% fetal bovine serum (FBS) (HyClone, GE Healthcare) and 30 μg/ml gentamicin. Cells were maintained at 37ºC in a humidified atmosphere in 5% CO_2(v/v).

Methods

The unit cell parameters of the monocrystal examples of complexes **III**, **IV**, **VII** and **VIII** were determined using single crystal diffractometer Bruker Smart APEX II equipped with a two-dimensional detector of the reflected x-rays type CCD (K radiation, λ = 0.71073 Å, graphite monochromator), in the resource centre "X-ray and diffraction methods research", St. Petersburg State University. Parameters of cell units were the same to published and registered structures in CCDC (Cambridge Crystallographic Data Centre).

Infrared spectra were recorded as KBr pellets 4000-450 cm^{-1} on a Shimadzu IRTracer-100 spectrometer. The bands are reported as s - strong, m - medium, sh - shoulder, b - broad, v - very.

The metal analysis was performed by gravimetric assay after reduction of the complexes at 800 °C in a stream of dry hydrogen. Element analyses for carbon, hydrogen and nitrogen presence was carried out using Perkin-Elmer 2400 CHNS-analyser. Quantitative determination of chlorine was carried out by potentiometric titration with silver nitrate solution without chloride ion, in the presence of nitric acid after removing sample complex with a tenfold weight excess of sodium carbonate at 800°C.

Carl Zeiss LSM 5 Pascal was used for observation for all cells in phase contrast and in fluorescence.

Colourimetric determination of optical density of solutions of formazan was proceeded by using multichannel spectrophotocolorimeter Fluorophot Charity (OOO “Probnauchpribor”, Russia).

Cytotoxicity analysis

Infusories

The complexes were dissolved in Losin-Losinski's buffer saline and diluted to the required concentration for cytotoxic study with *Paramecium caudatum.* Cells were washed by centrifugation (800 rpm, 2 min) in Losin-Losinski's buffer saline, live infusories were seeded quadruplicate in 96-well plate in 50 μl per well and incubated for 3 h at 25ºC. Then cells were counted by optical microscopy and were treated by the complex with determined concentration. After 6 h infusories were counted again by an optical microscope.

Human cell lines

The complexes were dissolved in phosphate buffered saline (PBS), sterilized by filtration (0,22μm, TPP, Switzerland) and diluted to the required concentration with serum-free culture medium (ranging from 10^{-3} to 10^{-9} M) for cytotoxic study with human cells. Serum-free medium was used to reduce interaction of metal complexes with proteins in FBS.

The effect of the complex on cell proliferation was determined by 3-[4,5-dimethyltiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [28]. A431 and HaCaT ($5\cdot10^4$ and $7\cdot10^4$ per ml, respectively) cells were seeded quadruplicate in 96-well plate (TPP, Switzerland) and incubated at 37 ºC in 5% CO_2 incubator for 12 hours. Then the medium was removed and cells were treated with the predefined fixed concentration of the complex in serum-free medium for 12 hours. Control wells were prepared by addition of DMEM without complexes (100% live cells) and Triton X-100 (10% per well; 100% death cells). Upon completion of the incubation, the medium was removed and MTT solution (5 mg/ml) was added. After 2 h incubation, 2-propanol (100 μl) with 0,04M HCl was added to dissolve formed formazan. The OD was measured at a wavelength of 570 nm minus 490 nm using a microplate reader. The IC_{50} value was determined from plots of % viability versus the dose of complexes added.

Statistical analysis

All statistical analyses were performed using the Sigma Plot 13 software for Windows. The significance was calculated using one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered statistically significant. Results are expressed as mean±SD.

Results and discussion

As an object of study, the choice of *Paramecium caudatum* is caused by a big size and high mobility of these cells. Such qualities of culture determine its wide use as a model organism in cyto- and protozoan investigations, especially as a test organism in toxicology and biotesting [29]. *P. caudatum* gives some specific responses for fast entering in its environment of a substance, so the toxicity degree can be easily evaluated [29,30]. During the study, we have noticed a few specific responses such as:

- Change of speed and path of movement;
- Positive or negative chemotaxis;
- Change of rhythm of the pulse of contractile vacuole or its paralysis;
- Formation of spherical intracellular structures unknown nature (blebbing of cells);
- Dyskinesia of flagellums;
- Activation of trichocysts;
- Stopping of cytokinesis;
- Changes in cell morphology.

Adding solutions of complexes **I-VIII** in a solution containing the culture *P. caudatum* immediately leads to negative chemotaxis, sharp speed increase of the cells and ciliary dyskinesia at any concentration.

By increasing the concentration and time of action of **I-VIII** in addition to the above phenomena, there is an increase in frequency and amplitude ripple contractile vacuoles trichocysts activation, deformation of the cell, and further -bloating and paralysis of contractile vacuole, vacuolation of the cytoplasm, the termination of cytokinesis, which ultimately leads to cell death (see Online Supplementary Materials, pp. 37, 38).

Steps of the cell death of *P. caudatum*, starting with the deformation of the body of infusoria, swelling of the contractile vacuole, formation of protrusions of the cytoplasmic membrane of the cell, bloating and paralysis of contractile vacuole, swelling of the cell, rupture of the cell membrane and release of cellular contents, and finally - complete lysis of cells (see Online Supplementary Materials, pp. 37, 38).

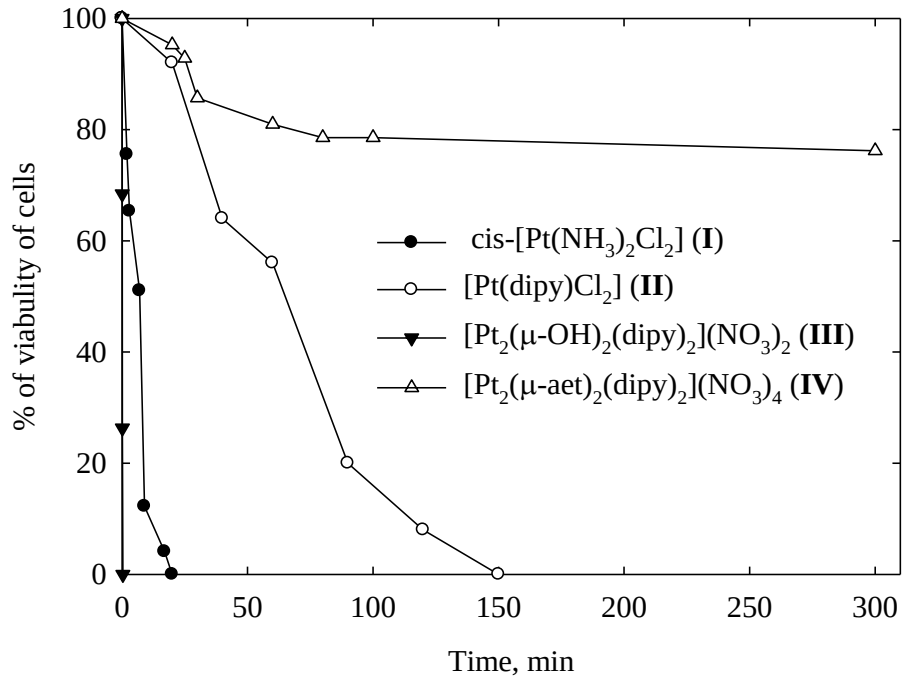


Figure 3. Survival curves of cells exposed to complexes of platinum versus time ($C_{I, II, IV}$ = 1 mM; C_{III} = 0,1 mM)

The most toxic to the *P. caudatum* are acid-ligands complexes (**I**, **V**), their hydrolysis and oligomerization products - binuclear hydroxido-bridge complexes **III** (Pt) and **VII** (Pd), and the least toxic - binuclear thiol-bridged complexes **IV** (Pt) and **VIII** (Pd). The effect is most pronounced in the case of complex **III**, where cell death occurs even at a concentration of 1 nM, whereas, in the presence of 1 mM **VIII** (Pd), **IV** (Pt) most of the cells survive even after 5 hours of exposure (Fig. 3). Relative cytotoxicity **I-VIII** on *P.caudatum*, HaCaT and A431 is given in Table. 1.

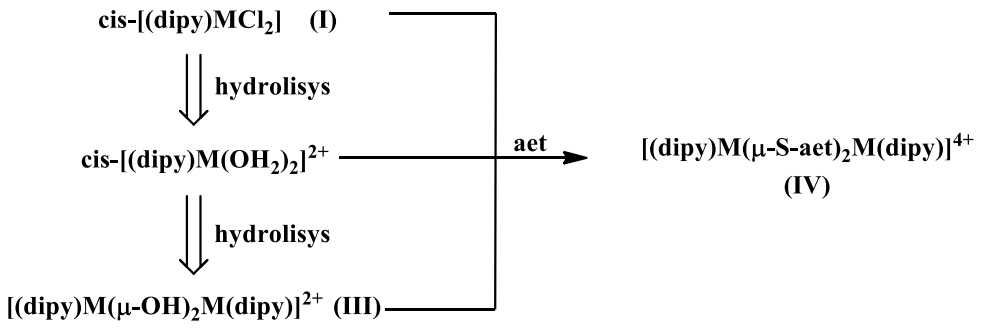
Table 1. Results of cytotoxicity of platinum and palladium complexes (IC_{50}, μM) on *P.caudatum*, A431 and HaCaT.

№	Complex/Cell line	A431	HaCaT
I	cis-$[Pt(NH_3)_2Cl_2]$	35.42±0.92	31.10±1.06
II	$[Pt(dipy)Cl_2]$	58.13±0.71	48.1±1.15
III	$[Pt_2(\mu\text{-}OH)_2(dipy)_2](NO_3)_2\cdot 2H_2O$	0.98±1.10	0.6±0.78
IV	$[Pt_2(\mu\text{-}S\text{-}aet)_2(dipy)_2](NO_3)_4\cdot 2H_2O$	189.52±1.21	152.14±0.93
V	cis-$[Pd(NH_3)_2Cl_2]$	25.42±0.97	11.9±1.01
VI	$[Pd(dipy)Cl_2]$	38.27±1.25	29.2±1.51
VII	$[Pd_2(\mu\text{-}OH)_2(dipy)_2](NO_3)_2\cdot 2H_2O$	0.02±0.85	0.009±0.84
VIII	$[Pd_2(\mu\text{-}S\text{-}aet)_2(dipy)_2](NO_3)_4$	132.91±1.18	124.85±0.75

The difference between cytotoxicity of **I-VIII** can be explained from different points of view. $Pt(dipy)Cl_2$ (**II**) complex is similar to *cis*-$[Pt(NH_3)_2Cl_2]$ (**I**), but including more steric and huge nitrogen heterocyclic ligand (dipy) hydrolyses by a similar mechanism, like the **I** do, forming the same products- $Pt(dipy)(OH_2)_2^{2+}$ and $Pt(dipy)(OH_2)Cl^{+}$. However, the process goes kinetically slow. Furthermore, the influence of diimine dipy-ligand, which **II** contains, is a more complicated ligand, following reduction of complex's toxicity at all or the display of «delayed» toxicity. It is important to note when acting **I** to infusoria cell culture, it shows us «irreversible» cell's death, morphologically alike to necrosis processes in eukaryotic cells. In that case, using **II**, *P. caudatum* species proceed to «stationary, inactive» condition under the low concentration of **II** (10^{-6} M), or death with the higher concentration (10^{-3} M). At $C_{complex} = 10^{-6}$ M until certain moment cells completely stop functioning and, partially, die. Nevertheless, vital functions of stopped cells have not stood still – cyclosis is observed, contractile vacuole continues to operate rhythmically, etc. In 12-14 hours *P. caudatum* species begin to «reactivate». 24 hours later all the infusoria completely restore their vital activity after undergoing toxic and, as a consequence, toxic stress [31], at the same time, defects inside the cell are quite perceptible with the microscope (cytoplasm vacuolization, in a less degree – macronucleus darkening) (see Online Supplementary Materials, pp. 37, 38).

For checking of a hypothesis of higher toxicity of hydrolytic polynuclear forms of platinum compounds in comparison with mononuclear ones, the experiment with a binuclear complex $[Pt_2(\mu\text{-}OH)_2(dipy)_2](NO_3)_2\cdot 2H_2O$ (**III**, Fig.2) has been made. At a concentration of **III** equal to 1 - 10 mM all the infusoria die instantly (~5 – 10 sec). At C = 10 μM total death take place under ~25 minutes (analogically with *cis*-$[Pt(NH_3)_2Cl_2]$ – 130 minutes), but even with three orders lower concentration (C = 10 nM) all cells permanently die. In that case, when **III** is initially entered into solution with cages, but it isn't formed as a result of difficult, multistage and reversible [11] processes of hydrolysis, the maximum speed of death of individuals of *P. caudatum* is observed. This fact can be explained by several variants, but agrees one of which - III is very reactive [14,32] and, as a result, damage of any biomolecules, first of all, of proteins occurs, whereas the topochemical site of coordination of a platinum complex of proteins isn't important.

The products of interaction of initial **I, II, V, VI** with simple model endogenic thiol – aet [33] (binuclear thiol-bridged complexes $[Pt_2(\mu\text{-}S\text{-}aet)_2(dipy)_2]^{4+}$ (**IV**, Fig. 2) и $[Pt_2(\mu\text{-}S\text{-}aet)_2(dipy)_2]^{4+}$ (**VIII**, Fig. 2)) are somewhat less toxic to all the cell types.



So, reviewing an example with **IV**, isostructural to **III**, the death of cells is observed only at extremely high concentrations of them in the system. At a concentration of 10 mM total death occurs approximately in the 5th minute, at 1 mM – only 20% die. As well as in a case with **II**, after "inactivation", the cells, which have undergone toxic shock "come to life" approximately, in 24 hours again. By data from [34] bi- or oligonuclear thiolato complexes, similar **IV** or **VIII**, are the main metabolites of both platinum complexes and some other d-elements. Complexes **I-III** and **V-VII** contacting endogenous thiols in an organism are inactivated and lose a possibility of damaging

various biomolecules, including proteins and DNA that probably explains the low toxicity of thiolato complexes **IV** and **VIII**.

It is considered that because of the bigger lability of palladium complexes [32] their inactivation happens *in vitro* significantly faster, than for the similar complexes of platinum, and, therefore, they are less toxic for a cell in general [35]. However, there is a number of works where it was shown that some compounds of palladium possess more expressed cytostatic action than similar complexes of platinum [36].

According to the modern observations` conclusion about the smaller toxicity of compounds of palladium in comparison with platinum, in general, coincides with the standard opinion [35,37]. However, it should be noted that the palladium-containing complex **VII** is more toxic in comparison with its platinum analogue **III**.

Interestingly, that the cells of *P. caudatum* processed by **I-III** and their palladium analogues (**V-VII)**, completely lose the ability to further proliferation, both mitotic, and sexual: when placing them on fresh Lozin-Lozinsky medium with *B. subtilis* as the power supply, it wasn't possible to receive posterity.

For confirmation of the toxicological data obtained on *P. caudatum*, a series of tests (MTT assay) on human cell lines A431 and HaCaT was held. Data of LD_{50} (Table 1) of complexes on these lines completely correlate with the obtained data on *P. caudatum*. Despite, the significantly high toxicity of connections **III** and **VII** should be noted. The explanation for this fact can be offered with taking into account a high reactionary reactivity of **III** and **VII**. The tests of survival carried out in serum-free DMEM, actually containing inactivators of these connections - cysteine and methionine, have shown that complexes **III** and **VII** are easily capable of forming chelate compounds [Pt(dipy)(N,S-Cys)] and [Pt(dipy)(N,S-Met)], passing into inactive forms. Nevertheless, despite it, they remain the most toxic among a platinum and palladium complexes.

Conclusion

The initial aminate cis-complexes of platinum(II) and palladium(II) do not possess high toxicity, while their hydrolysis products do. With the other things being equal, oligonuclear hydrolytic forms of platinum complexes are on two orders more toxic than original ones. Among the *in vivo* formed aminate cis-complexes of platinum(II) and palladium(II) the hydroxy-bridged bi- and oligonuclear forms show the most toxic abilities, which are formed during hydrolysis processes, the least – the analogical thiolato forms, emerging from an interaction of hydrolytic complexes with an endogenous thiol. Generally, the toxicity of complexes (M = Pd(II), Pt(II)) increases in the row: $[M_2(\mu\text{-S-aet})_2(dipy)_2]^{4+}$ << $[M(dipy)Cl_2]$ < cis-$[M(NH_3)_2Cl_2]$ << $[M_2(\mu\text{-OH})_2(dipy)_2]^{2+}$. It is worth noting, that, under equal conditions hydrolysis forms of complexes initiate necrosis in the much smaller amount of cells in relation to cisplatin and could stand the racket on high nephro- and ototoxicity.

The appearance of temporary «inactivation» of P.caudatum species under the influence of low concentrations (<10μM) of $[M(dipy)Cl_2]$ complexes was observed: there was a suspense in a species' motion, also the dyskinesia of cilia and contractile vacuole's inflation was discovered. After a while (12-18 hours) species «activated» again and returned to the vital activity.

As a result, to sum up, all the said above, it is recommended to use cisplatin its analogues either as powder, obtained by lyophilisation, diluted directly before application or as an acid buffer solution (pH ≤ 4,5 - 5). In both cases, the hydrolysis and cis-trans complexes isomerization reactions in solution are prevented or significantly decreases, in the case of smaller values of pH of the solutions the formation of highly oligonuclear hydrolysis forms is suppressed.

Acknowledgment

The authors appreciate the constructive critique and given materials and cell lines of A. V. Popov, prof. D. O. Vinokchodov and M. V. Pushkarev from Saint-Petersburg State Institute of Technology (Technical University), and Dr. E. Y. Komarova, Dr. I. V. Guzhova and Dr. B. A. Margulis from Institute of Cytology Russian Academy of Sciences.

This work was supported partly by Ministry of Education and Science of Russia (4.4697.2017/6.7), by Russian Foundation for Basic Research (16-08-01299) and by Grant of Russian Science Foundation (RSCF) No 14-50-00068 (IG, BM).

Conflict of Interest

The authors declare no conflict of interest.

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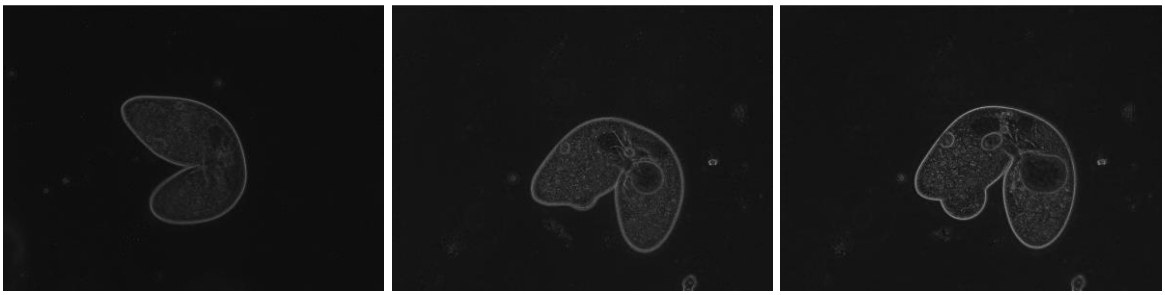
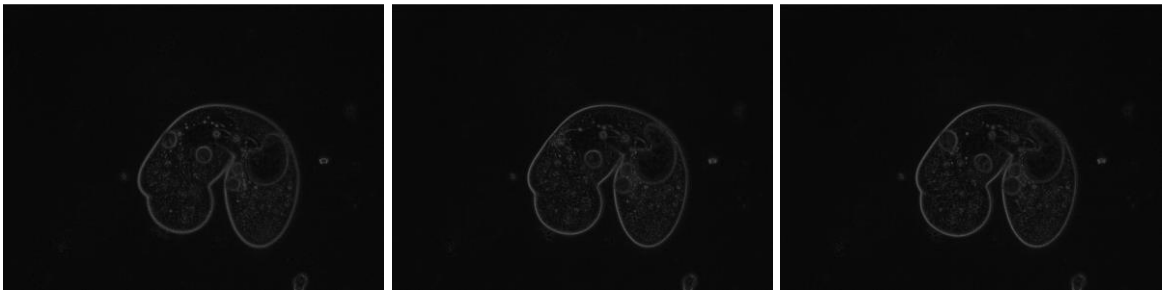
Supplementary materials



a



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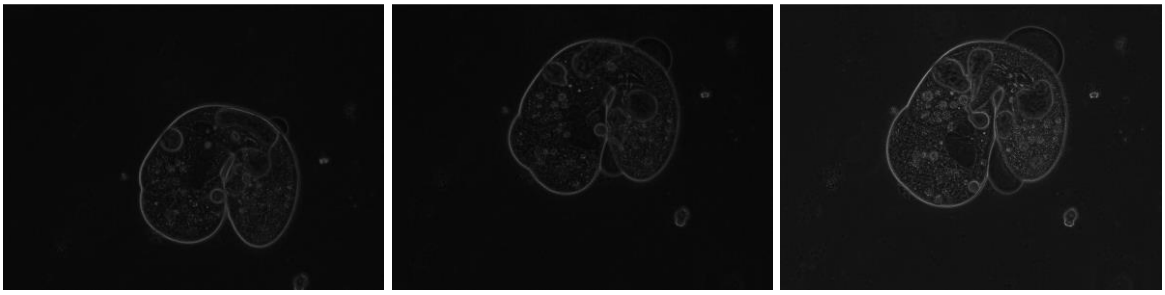

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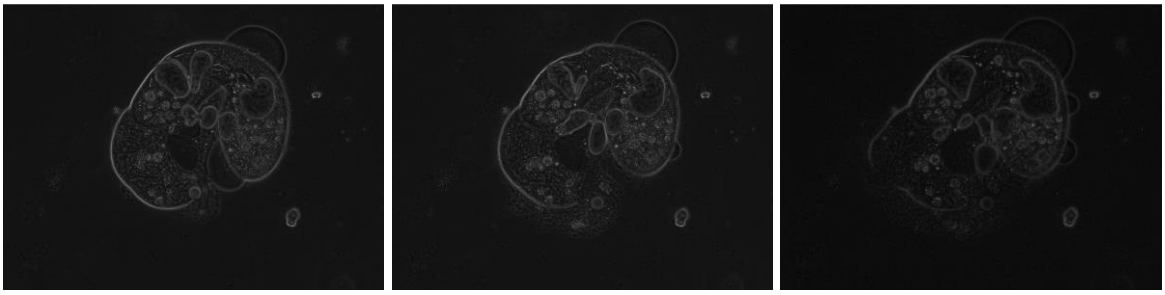

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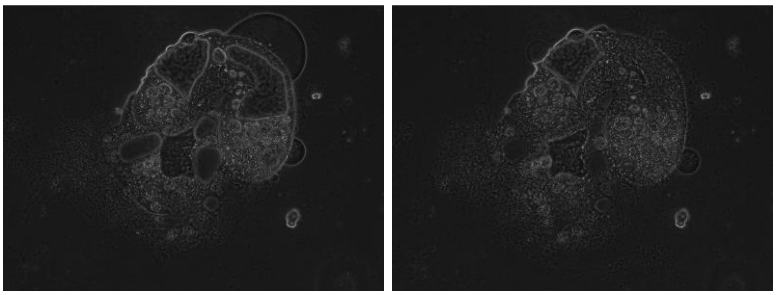

e

Fig. 4. Steps of death of *P. caudatum.*

Block **a** - deformation of the body of infusoria and swelling of the contractile vacuole.

Block **b** - swelling of the cell; vacuolation of the cytoplasm.

Block **c** - bloating and paralysis of contractile vacuole; membrane`s blebbing.

Block **d** - formation of protrusions of the cytoplasmic membrane of the cell, termination of cytokinesis.

Block **e** - rupture of the cell membrane and release of cellular contents, complete lysis of cells.