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Pt(IV), Pd(II), and Rh(III) complexes induced oxidative stress and cytotoxicity in the HCT-116 colon cancer cell line

Petar ČANOVIĆ[1], Jovana BOGOJESKI[2], Jelena V. KOŠARIĆ[3], Snežana D. MARKOVIĆ[3], Marko N. ŽIVANOVIĆ[3,*]

[1]Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia

[2]Department of Chemistry, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

[3]Department of Biology and Ecology, Faculty of Science, University of Kragujevac, Kragujevac, Serbia

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Abstract: The use of transition metal complexes as appropriate analogues to cisplatin and similar anticancer drugs nowadays is of utmost importance. The inertness of Pt(IV), Pd(II), and Rh(III) complexes and the choosing of convenient ligands could offer a possible advantage in comparison to Pt(II) complexes. These advantages could be attributed to the better anticancer activity and fewer possible side effects. In this paper, we have chosen three complexes of different transition metal ions, $[(TL^{tBu})Pd^{II}Cl]ClO_4$, $[Rh^{III}(LH_2^{tBu})Cl_3]$, and $[Pt^{IV}(LH_2^{tBu})Cl_3]Cl$ (where TL^{tBu} is 2,6-bis[(1,3-di-tert-butylimidazolin-2-imino)methyl]pyridine and LH_2^{tBu} is 2,6-bis(5-tert-butyl-1H-pyrazol-3-yl)pyridine), for biological tests. All three complexes exerted strong prooxidative and cytotoxic effects on the human colorectal colon cancer HCT-116 cell line. The Pd(II) complex showed the most significant effect, while the Rh(III) complex showed the least effect.

Key words: Anticancer activity, colon cancer, cytotoxicity, oxidative stress, palladium, platinum, rhodium

1. Introduction

Cancer is the second leading cause of death after cardiovascular diseases, and finding the appropriate therapy is of key medical and scientific interest, with a potentially substantial economic impact. There are about 200 different types of cancer of various incidences; some are very common while others are rare (Penny and Wallace, 2015). All types of cancer display a characteristic uncontrolled cell division followed by the ability of these cells to invade healthy tissues. The development of cancer involves a series of complex mechanisms related to the individual's genetic characteristics and exposure to certain risk factors. Understanding of the targets and mechanisms of action of potential therapeutics in cancer cells is essential for drug design and clinical applications (Bergamo and Sava, 2015; Penny and Wallace, 2015).

Cisplatin was the first used metal-based drug in the world. Although more than 40 years have passed since the discovery of cisplatin's antitumor properties, the platinum drugs are the only metal-based drugs used in clinical practice for the treatment of cancer. The advantages and drawbacks of the widely used platinum-based anticancer drug cisplatin prompted a search for analogous transition metal complexes. One of the major goals of modern bioinorganic and medicinal chemistry research is the development of novel metal-based drugs with pharmaceutical activity different from that of platinum-based therapeutics (Lippert, 1999; Alessio, 2011; Bergamo and Sava, 2015).

Initially it was assumed that the palladium(II) complexes do not possess antitumor properties and, compared to cisplatin, the corresponding cispalladium complex, *cis*-$[Pd(NH_3)_2Cl_2]$, does not show antitumor activity. Since the coordination chemistry of Pd(II) and Pt(II) compounds is very similar, the Pd(II) complexes were very often used as model molecules since they react about 104–105 times faster than their Pt(II) analogues, whereas the structural and equilibrium behaviors of the solutions are very similar (Bugarčić et al., 2015). However, the introduction of some spectator ligands in the coordination sphere of Pd(II) can result in complexes with significant antitumor properties (Abu-Surrah and Kettunen, 2006; Gao et al., 2008, 2009).

The main strategy in the designing of new metal-based drugs is to create drugs that will be sufficiently reactive to bind to the biological target, but not so reactive that they will be deactivated by other biomolecules different from the target; this can be achieved as a prodrug. A prodrug is a compound that can undergo a transformation in vivo

* Correspondence: zivanovicm@kg.ac.rs

to release an active substance that could act as a drug in situ. The Pt(IV) complexes are the most famous group of prodrugs that can be used to overcome some of the problems associated with cisplatin and its analogues. The high kinetic inertness of platinum(IV) complexes relative to their platinum(II) analogues introduces drug stability and the two extra ligands on the octahedral metal center offer many possibilities for modification of pharmacokinetic parameters (Ronconi and Sadler, 2007; Barry and Sadler, 2013).

Today's research in the field of metal-based drugs is also directed towards metal complexes from the 9th group of the periodic table, iridium, osmium, and rhodium complexes (Petrovic et al., 2008; Bogojeski et al., 2011; Yoshinari et al., 2012). The main feature of these complexes is inertness. However, the reactivity and biological activity of these complexes can be improved by a suitable choice of spectator ligands.

Recently we showed that the choosing of appropriate ligands could provide palladium(II) complexes that are extremely cytotoxic to cancer cells (Petrovic et al., 2015). Under standard laboratory conditions for in vitro testing, it was shown that those Pd(II) complexes produced extremely high levels of reactive radical species and that could be toxic to cancer cells, even more so than cisplatin. In the presented work we have used the Pd(II), Pt(IV), and Rh(III) complexes for the in vitro testing of cell viability and oxidative stress on the human colorectal carcinoma cell line HCT-116.

2. Experimental

2.1. Material and methods

The K_2PdCl_4, Na_2PtCl_6, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO, NBT (nitro blue tetrazolium chloride), NED (N-1-naphthyl ethylenediamine dihydrochloride), sulfanilamide, phosphoric acid, DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), EDTA, sulfosalicylic acid, monosodium phosphate, and disodium phosphate were obtained from Sigma Aldrich (St. Louis, MO, USA). DMEM (Dulbecco's modified Eagle medium), FBS (fetal bovine serum), PBS (phosphate-buffered saline), trypsin-EDTA, and penicillin/streptomycin were from Thermo Fisher Scientific (Waltham, MA, USA). The ligands TL^{tBu} (2,6-bis[(1,3-di-*tert*-butylimidazolin-2-imino)methyl] pyridine) and LH_2^{tBu} (2,6-bis(5-*tert*-butyl-1H-pyrazol-3-yl)pyridine) were prepared according to published procedures (Petrovic et al., 2008; Yoshinari et al., 2012). The complexes $[(TL^{tBu})PdCl]^+$ and $[Rh(LH_2^{tBu})Cl_3]$ were also prepared according to prior procedures (Bogojeski et al., 2011; Milutinović et al., unpublished data). All the other chemicals were of the highest purity commercially available.

2.1.1. Synthesis and characterization of $[Pt(LH_2^{tBu})Cl_3]Cl$ complex

To a solution of 100 mg (0.220 mmol; 1 eq.) of Na_2PtCl_6 in 50 mL of water was added 71 mg (0.220 mmol; 1 eq.) of ligand LH_2^{tBu} in 10 mL of water. The reaction mixture was stirred overnight at 50 °C, affording a yellow precipitate. The pH of the solution was around 5.0. The precipitate was filtered and dried in vacuo. The product was obtained as a yellow solid (125 mg, 84.13%).

^{1}H NMR (d_6-DMSO): δ = 1.96 (s, 18H, CMe_3), 7.37 (s, 2H; pyrazole), 8.34 (d, 2H, pyridine), 8.55 (t, 1H, pyridine) ppm. ^{13}C NMR (100 MHz; $CDCl_3$): δ = 29.9 (s, $C\underline{C}H_3$), 33.0 (s, $\underline{C}CH_3$), 102.8 (s, 4C, pyrazole), 126.0 (s, 2C, pyridine), 148.3 (s, 1C, pyridine), 152.9 (s, 2C, pyrazole), 153.6 (s, 2C, pyridine), 154.2 (s, 2C, pyridine) ppm. IR (KBr, 4000–300 cm^{-1}): 3447 (N-H stretch); 2984, 2927, 2852 (C-H stretch), 1634, 1574, 1483 (C=C and C=N stretch); 625 (Pt-N stretch). Anal. Calcd. for ($C_{20}H_{28}Cl_4N_5Pt$) C: 35.57; H: 4.18; N: 10.37. Found: C: 36.95; H: 4.35; N: 11.06.

2.1.2. Characterization of $[(TL^{tBu})PdCl]ClO_4$ complex

The complexes $[(TL^{tBu})PdCl]^+$ and $[Rh(LH_2^{tBu})Cl_3]$ were prepared according to published procedures (Bogojeski et al., 2011).

^{1}H NMR (200 MHz, D_2O): δ = 7.56 (2 H, d, *m*-Py), 7.45 (1 H, t, *p*-Py), 4.57 (4 H, s, Py-CH_2), and 1.68 (36 H, s, CCH_3). Anal. Calcd. for $C_{30}H_{52}Cl_2N_7O_4Pd$: C, 47.9; H, 6.9; N, 13.0. Found: C, 47.8; H, 6.2; N, 13.5.

2.1.3. Characterization of $[Rh(LH_2^{tBu})Cl_3]$ complex

The complex $[Rh(LH_2^{tBu})Cl_3]$ was prepared according to prior procedures (Milutinović et al., unpublished data).

^{1}H NMR (d_6-DMSO): δ = 8.19 (d, 2H, $^3J_{HH}$ = 7.6 Hz, pyridine), 7.30 (t, 1H, $^3J_{HH}$ = 7.6 Hz, pyridine), 7.22 (s, 2H, pyrazole), 2.47 (s, 18H, CMe_3) ppm. Anal. Calcd. for ($C_{19}H_{25}Cl_3N_5Rh\bullet1.25H_2O$) C: 41.10; H: 4.99; N: 12.61. Found: C: 40.95; H: 4.85; N: 12.96. IR (KBr, 4000–300 cm^{-1}): 3466 (N-H stretch); 2924 (C-H stretch); 1623, 1574, 1442 (C=C and C=N stretch); 635 (Rh-N stretch).

2.1.4. Cell preparation and culturing

The human colorectal cancer cell line HCT-116 was purchased from the American Tissue Culture Collection (Manassas, VA, USA). The cell cultures were maintained under controlled conditions in a humidified atmosphere with 5% CO_2 at 37 °C in 75-cm^2 culture flasks. The cells were grown in complete medium (DMEM supplemented with 10% FBS and 100 IU/mL penicillin and 100 µg/mL streptomycin) and seeded for experimental purposes at 70%–80% cell confluence.

2.1.5. MTT assay for cell viability

The MTT tetrazolium reaction assay is based on the colored reduction of yellow MTT to purple formazan in living cells (Mosmann, 1983). This color change corresponds to the activity of mitochondria and could be

subsequently measured at wavelengths between 550 and 600 nm. In the MTT assay 10^4 cells were seeded in 96-well plates. After 24 h, the cells were treated with 100 µL of investigated substances in a final concentration range from 0.1 to 500 µM. Cell viability was estimated 24 and 72 h after treatment by replacing the medium containing treatment substances with 100 µL of medium supplemented with 25 µL of MTT (concentration of 5 mg/mL PBS). After 3 h of tetrazolium reduction reaction (in controlled conditions of 37 °C, 5% CO_2), MTT-containing medium was replaced with 150 µL of DMSO. After 15 min, purple formazan was well dissolved and absorbance was measured. Untreated cells served as a negative control, while cell viability was expressed as the ratio of measured absorbance of treated and control cells multiplied by 100 to obtain a percentage. A plot of % cell viability against substance concentrations was used to calculate the concentration that showed 50% cytotoxicity (IC_{50}).

2.1.6. NBT assay: determination of superoxide anion radical

The spectrophotometric assay for determining the superoxide anion radical, $O_2^{\bullet-}$, is based on measuring the absorbance of nitroblue-formazan resulting from the reduction of NBT in the presence of $O_2^{\bullet-}$ (Auclair and Voisin, 1985) at 550–600 nm. In this assay, 10^4 cells were seeded in 96-well plates and after 24 h were treated with 100 µL of the investigated substances in a final concentration range from 0.1 to 500 µM. The $O_2^{\bullet-}$ was estimated 24 and 72 h after treatment by adding 10 µL of NBT (5 mg/mL PBS), incubated under controlled conditions for 45 min with subsequent measuring of absorbance. Untreated cells served as a negative control.

2.1.7. Griess assay: determination of nitrites

Spectrophotometric detection of nitrites, which could be considered as indicators of nitric oxide (Griess, 1879), is based on diazotation reaction of nitrites and sulfanilamide and NED. A purple diazo product could be spectrophotometrically detected at 520–550 nm. Similarly to the MTT and NBT assay, 10^4 cells were seeded in 96-well plates with subsequent treatment and after 24 and 72 h nitrite measuring was performed. The procedure involves using 50 µL of supernatant from 96-well plates, adding an additional 50 µL of freshly prepared 1% sulfanilamide (dissolved in 5% phosphoric acid) and 50 µL of freshly prepared 0.1% NED solution. Such a prepared reaction mixture is measured on an ELISA reader, and untreated cells were considered as a control.

2.1.8. GSH assay: determination of reduced glutathione

The GSH assay is a spectrophotometric method based on oxidation of the reduced form of glutathione with DTNB, where a yellow product, 5′-thio-2-nitrobenzoic acid, is measured at 405 nm (Baker et al., 1990). In this assay, 5×10^4 cells were seeded in 96-well plates and treated in the same manner as in the MTT, NBT and Griess assays. First they were disintegrated with 2.5% ice-cold sulfosalicylic acid, and then 50 µL of supernatant was mixed with 100 µL of reaction mixture consisting of 100 mM phosphate-EDTA buffer, pH 7.4, and 1 mM DTNB, followed by measuring of absorbance on an ELISA reader. Untreated cells were considered as a control.

2.2. Instrumentation

UV-VIS spectra were recorded on PerkinElmer Lambda 35 spectrophotometers with thermostated 1.00-cm quartz Suprasil cells. ^{1}H NMR measurements were performed on a Varian Gemini 2000 NMR spectrometer (200 MHz). Chemical analyses were performed on a Carlo Erba elemental analyzer, model 1106. The IR spectra were recorded on a PerkinElmer FTIR 31725. Spectrophotometric analyses of reactive species were conducted on a Rayto 96-well plate ELISA reader, RT-2100C.

2.3. Statistics

All experiments were performed in triplicates for each dose, and data are presented as mean ± standard error (SE). Statistical significance was determined using the one-way ANOVA test for multiple comparisons. $P < 0.05$ was considered as significant. The magnitude of correlation between variables was determined using SPSS 17 for Windows (SPSS Inc., Chicago, IL, USA). The IC50 values were calculated from the dose curves by a computer program (CalcuSyn).

3. Results

3.1. Complex synthesis

The Pt(IV) complex (Figure 1) was synthesized by stirring equimolar amounts of Na_2PtCl_6 and the 2,6-bis(5-*tert*-butyl-1H-pyrazol-3-yl)pyridine ligands in water at a pH of around 5. The obtained Pt(IV) complex was characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis, and IR spectroscopy.

In the ^{1}H NMR and ^{13}C NMR spectra of the $[Pt(LH_2^{tBu})Cl_3]^+$ complex signals were detected from tert-butyl groups, pyridine ring protons, and protons of pyrazoles, which were significantly shifted compared to the free ligand. In the IR spectrum of the $[Pt(LH_2^{tBu})Cl_3]Cl$ complex it can be seen that there is a strong signal at about 3447 cm^{-1} corresponding to valence vibration of N-H bonds. In the area of 2984–2852 cm^{-1} there are bands that originate from symmetric valence vibration of C-H bonds. In the area of 1600–1400 cm^{-1} are bands derived from aromatic valence vibration of the C=C double bond as well as the vibration of the C=N bond. At 625 cm^{-1} there is a signal from the valence vibration of the Pt-N bond.

The complexes $[(TL^{tBu})PdCl]^+$ and $[Rh(LH_2^{tBu})Cl_3]$ were prepared according to prior procedures (Bogojeski et al., 2011; Milutinović et al., unpublished data). The purity

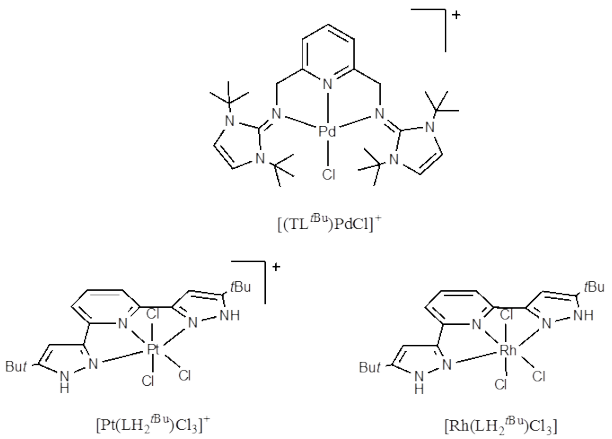


Figure 1. The structures of studied complexes along with observation.

of the complexes was checked by NMR and elemental analysis and good agreement was obtained.

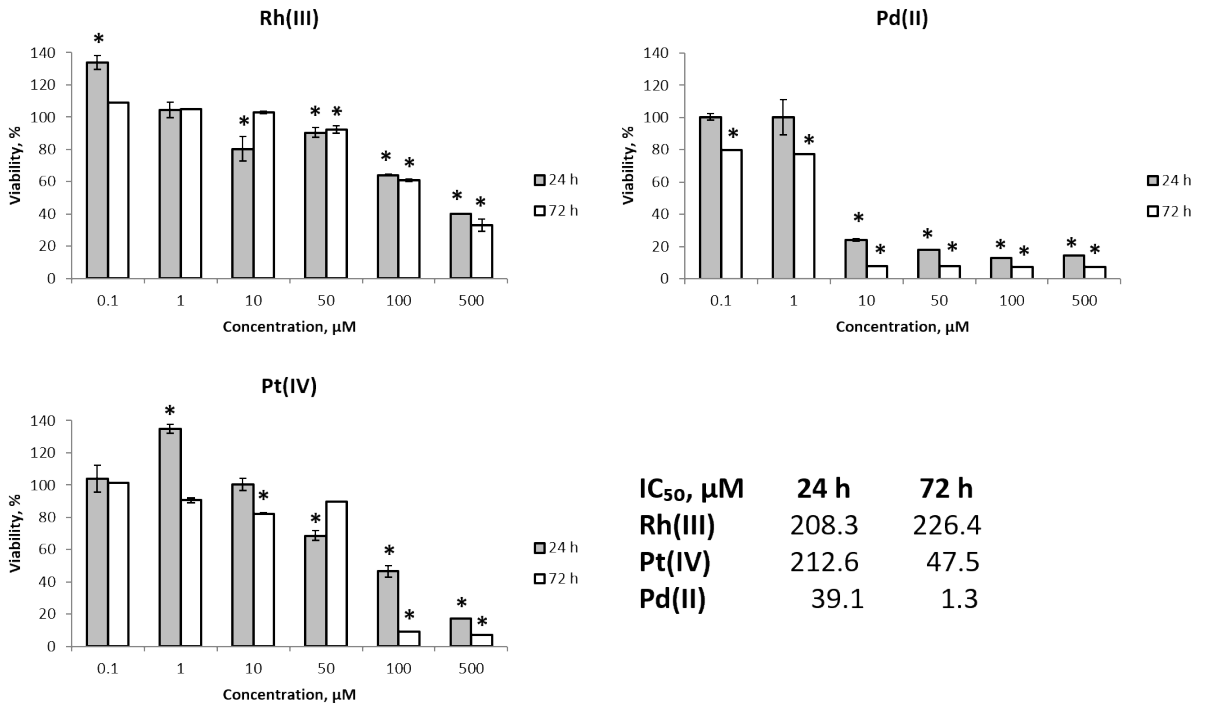
3.2. Biological evaluation

3.2.1. Cytotoxicity of Rh(III), Pt(IV), and Pd(II) complexes

Cytotoxicity results of the investigated complexes are depicted in Figure 2. We found that Pd(II) exerted the most significant cytotoxic effect with very low IC_{50} values (39.1 and 1.3 μM for 24 h and 72 h after treatment). Such a denominated cytotoxic character could be compared to the effect of the most cytotoxic substances, e.g., cisplatin, which under our laboratory conditions showed lower cytotoxic effects on HCT-116 cells with IC_{50} values of 255 and 29 μM measured 24 and 72 h after treatment (Petrovic et al., 2015).

3.2.2. Rh(III), Pt(IV), and Pd(II) complexes induced oxidative stress in HCT-116 cells

The levels of superoxide anion radical and nitrites were determined. Our results indicated a positive correlation between radical species generation and cytotoxic cell death: the more the increase of radicals, the greater the cytotoxicity. The most significant cytotoxicity was observed with Pd(II). Such cytotoxicity correlates to the level of measured $O_2^{\bullet-}$. The level of $O_2^{\bullet-}$ induced by Pd(II) is the greatest in comparison to Pt(IV) and Rh(III) (Figure 3). After 24 h from treatment, the $O_2^{\bullet-}$ level for Pt(IV) and Rh(III) was similar; thus, the cytotoxicity of these two substances was also similar. On the other hand, 72 h after treatment Pt(IV) induced much greater production of $O_2^{\bullet-}$ than Rh(III), which could also be followed by greater cytotoxicity of Pt(IV). It is interesting that Rh(III) induced similar production of $O_2^{\bullet-}$ after 24 and 72 h from treatment, and as one can observe, the cytotoxicity is quite similar, with IC_{50} ranging from 208 to 226 μM.



IC_{50}, μM	24 h	72 h
Rh(III)	208.3	226.4
Pt(IV)	212.6	47.5
Pd(II)	39.1	1.3

Figure 2. The dose response curves of the effects of Rh(III), Pt(IV), and Pd(II) on HCT-116 cell growth after 24 and 72 h of exposure. The antiproliferative effect was measured by MTT assay. All values are mean ± standard error, n = 3, *P < 0.05 as compared with control.

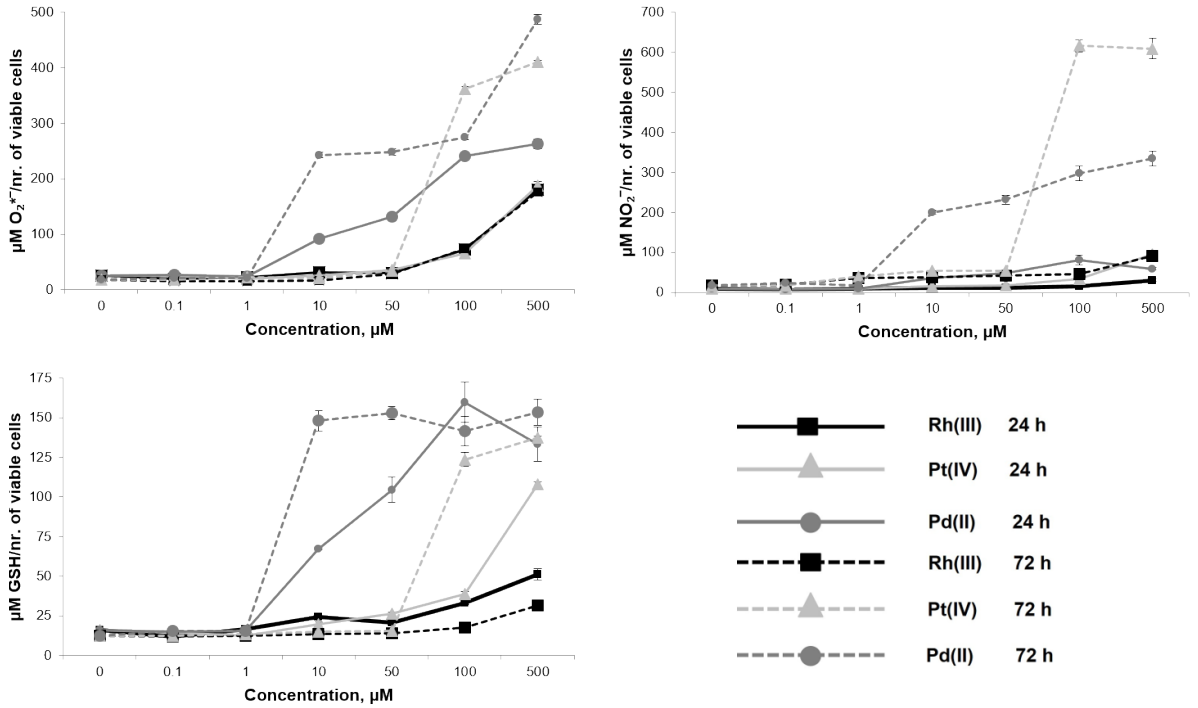


Figure 3. Effects of Rh(III), Pt(IV), and Pd(II) on HCT-116 cells, expressed as the $O_2^{\bullet-}$, NO_2^-, and GSH concentrations related to the number of viable cells after 24 and 72 h of exposure. All values are mean ± standard error, n = 3.

The fact that increased oxidative stress induces cell death is largely used for production of drugs with anticancer activity (Barrera, 2012). Besides the increasing in $O_2^{\bullet-}$ it is also very important to investigate reactive nitrogen species (RNS), which could be followed by measuring nitrites as NO indicators. Our results show that nitrites also increased as well as $O_2^{\bullet-}$, especially for Pd(II) and Pt(IV) (Figure 3). Rh(III) induced only slightly increasing nitrites in the highest concentrations, Pd(II) induced greatly increasing nitrites, and Pt(IV) induced the most significantly increasing nitrites.

In order to understand the influence of the investigated complexes on redox balance affected by increased production of $O_2^{\bullet-}$ and nitrites, we examined the level of GSH. Our investigation showed a significant increase in GSH level in the following order: Pd(II) > Pt(IV) > Rh(III). The increase for all three complexes is statistically significant, as shown in Figure 3.

4. Discussion

As demonstrated by previous studies, complexes of transition metals in many cases showed great cytotoxic effect on the HCT-116 cell line (Antonarakis and Emadi, 2010; Volarevic et al., 2013). We were interested to see whether these complexes would show similar behaviors and what the differences between them would be. Our complexes showed great cytotoxic effect against the HCT-116 cell line. The cytotoxicity induced by the Pd(II) complex was the greatest; Pt(IV) showed less effect, while Rh(III) exerted a smaller cytotoxic effect. Similar results of comparison of these three transition metal complexes were shown by Schmid et al. (2007), where the Rh(III) complex exerted little influence on human bronchial epithelial cells, while the Pd(II) and Pt(IV) complexes showed much greater cytotoxic effect.

Our results showed a strong relationship between cell viability and levels of superoxide anion radical and nitrites in the studied cell line. Many papers showed that cellular processes and cellular compartments were affected, leading in many cases to cell death, with the increasing of reactive oxygen species (ROS) and RNS (Deavall, 2012). The correlation between the level of ROS/RNS and the cytotoxicity of the investigated substances is presented in Figure 3. Such increasing of $O_2^{\bullet-}$ could be explained by the Fenton reaction inside the cells. Namely, H_2O_2 present in cells reacts with iron, yielding $O_2^{\bullet-}$. Similarly, the Fenton reaction could also be catalyzed by other transition metals, such as palladium, platinum, or rhodium (Halliwell and Gutteridge, 2007). Nitrites could also correlate with the cytotoxicity of some substances, but the level of nitrites (i.e. of NO) greatly depends on $O_2^{\bullet-}$ production. $O_2^{\bullet-}$ possesses a great affinity towards NO, forming very reactive peroxynitrites (Ferrer-Sueta and Radi, 2009), which could induce a lowering of the content of produced NO. It is also

quite complicated to explain the nitrite level. The content of nitrites could depend on available nitrogen-carrying compounds in cells that could be catabolized, especially when we realize that the ligands of our complexes possess many nitrogen atoms in imidazole and pyridine rings. On the other hand, enhanced activity of iNOS protein could also contribute to the production of NO. Our novel results suggest that the similar Pd(II) complex actually greatly increased nitrites by activation of iNOS protein, whose induction was detected by immunofluorescent microscopy assay (Živanović et al., unpublished data). Similarly to $O_2^{\bullet-}$ production, nitrite production increased in a dose- and time-dependent manner.

Usually the first reflection of increased oxidative stress is the production of GSH. GSH is a tripeptide responsible for the first defense of cells, keeping the redox equilibrium stable. De novo synthesis of GSH in our experiments could be explained in two ways. First, the different chemical compositions of the investigated substances could differentially interact with and activate some of the enzymes in cells that are responsible for GSH synthesis. The activation of GSH-producing enzymes could be considered as antioxidative behavior. Having in mind the increased $O_2^{\bullet-}$ and nitrites, one could conclude both antioxidative and prooxidative behavior. Second, substances induced increasing $O_2^{\bullet-}$ and nitrites, whose higher production subsequently led to the activation of cellular mechanisms for maintenance of redox equilibrium. This explanation is more likely, because it was proven that increased ROS/RNS induce activation of the thioredoxin system, which reduces GSSG to GSH prior to maintaining the redox equilibrium unchanged in rate (Nordberg and Arner, 2001). This led us to conclude that the investigated substances are rather prooxidative and thus cytotoxic to HCT-116 cells. On the other hand, influence on GSH-producing enzymes still remains only a possibility, and more work is needed to elucidate this concrete model of action of Pd(II), Pt(IV), and Rh(III).

In summary, we found that the investigated complexes increased oxidative stress on HCT-116 cells, which caused increased cytotoxicity. Pd(II) showed the most significant cytotoxicity and increase of superoxide anion and nitrites, Pt(IV) also showed considerable prooxidative and cytotoxic effects, and Rh(III) showed little influence on the HCT-116 cancer cell line.

Activity of the most active Pd(II) can be partly attributed to pyridine moiety in the used ligands. Similar, pyridine-based ligands showed antitumor activity against various types of cancer cells. 2,2′-Bipyridine complexed to Pd(II) showed great anticancer activity against human leukemic K562 cells (Disalvar et al., 2011). Similarly, terpyridine complexed to Pd(II) revealed significant activity against MDA-MB-231 and MCF-7 breast cancer cell lines (Ulukaya et al., 2011a) and A549, H1299, and PC-3 nonsmall-cell lung cancer cell lines (Ulukaya et al., 2011b) with IC_{50} values comparable to our results obtained on the colorectal cancer line HCT-116 with Pd(II).

According to the literature (Ulukaya et al., 2011a), substances with structures similar to that of the investigated Pd(II) proved to be very effective in experiments performed in vivo. Such substances could be seriously considered for further, more detailed analyses. Thus, one of goals we aim to achieve is continuing our investigations towards in vivo testing.

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References

Abu-Surrah AS, Kettunen M (2006). Platinum group antitumor chemistry: design and development of new anticancer drugs complementary to cisplatin. Curr Med Chem 13: 1337-1357.

Alessio E (2011). Bioinorganic Medicinal Chemistry. Weinheim, Germany: Wiley-VCH.

Antonarakis ES, Emadi A (2010). Ruthenium-based chemotherapeutics: are they ready for prime time? Cancer Chemother Pharmacol 66: 1-9.

Auclair C, Voisin E (1985). Nitrobluetetrazolium reduction. In: Greenwald, RA, editor. Handbook of Methods for Oxygen Radical Research. Boca Raton, FL, USA: CRC Press, pp. 122-132.

Baker MA, Cerniglia GJ, Zaman A (1990). Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Anal Biochem 190: 360-365.

Barrera G (2012). Oxidative stress and lipid peroxidation products in cancer progression and therapy. ISRN Oncol 2012: 137289.

Barry NPE, Sadler PJ (2013). Exploration of the medical periodic table: towards new targets. Chem Comm 49: 5106-5131.

Bergamo A, Sava G (2015). Linking the future of anticancer metal-complexes to the therapy of tumour metastases. Chem Soc Rev 44: 8818-8835.

Bogojeski J, Jelic R, Petrovic D, Herdtweck E, Jones PG, Tamm M, Bugarcic ZD (2011). Equilibrium studies of the reactions of palladium(II) bis(imidazolin-2-imine) complexes with biologically relevant nucleophiles. The crystal structures of $[(TLtBu)PdCl]ClO_4$ and $[(BLiPr)PdCl_2]$. Dalton Trans 40: 6515-6523.

Bugarčić ŽD, Bogojeski J, van Eldik R (2015). Kinetics, mechanism and equilibrium studies on the substitution reactions of Pd(II) in reference to Pt(II) complexes with bio-molecules. Coord Chem Rev 292: 91-106.

Deavall DG, Martin EA, Horner JM, Roberts R (2012). Drug-induced oxidative stress and toxicity. J Toxicol 2012: 1-13.

Disalvar A, Saboury AA, Ahadi L, Zemanatiyar E, Mansouri-Torshizi H, Ajloo D, Sarma RH (2011). Biological evaluation and interaction of a newly designed anti-cancer Pd(II) complex and human serum albumin. J Biomol Struct Dyn 29: 283-296.

Ferrer-Sueta G, Radi R (2009). Chemical biology of peroxynitrite: kinetics, diffusion, and radicals. ACS Chem Biol 4: 161-177.

Gao E, Liu C, Zhu M, Lin H, Wu Q, Liu L (2009). Current development of Pd(II) complexes as potential antitumor agents. Anticancer Agents Med Chem 9: 356-368.

Gao E, Zhu M, Yin H, Liu L, Wu Q, Sun Y (2008). Synthesis, characterization, interaction with DNA and cytotoxicity in vitro of dinuclear Pd(II) and Pt(II) complexes dibridged by 2,2′-azanediyldibenzoic acid. J Inorg Biochem 102: 1958-1964.

Griess P (1879). Bemerkungen zu der Abhandlung der HH. Weselsky und Benedikt "Ueber einige Azoverbindungen". Ber Dtsch Chem Ges 12: 426-428 (in German).

Halliwell B, Gutteridge (2007). Free Radicals in Biology and Medicine. New York, NY, USA: Oxford University Press.

Lippert B (1999). Cisplatin: Chemistry and Biochemistry of a Leading Antitumor Drug. Zurich, Switzerland: Wiley-VCH.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

Nordberg J, Arner ES (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radic Biol Med 31: 1287-1312.

Penny LK, Wallace HM (2015). The challenges for cancer chemoprevention. Chem Soc Rev 44: 8836-8847.

Petrovic D, Hill LMR, Jones PG, Tolman WB, Tamm M (2008). Synthesis and reactivity of copper(I) complexes with an ethylene-bridged bis(imidazolin-2-imine) ligand. Dalton Trans 21: 887-894.

Petrovic VP, Zivanovic MN, Simijonovic D, Dorovic J, Petrovic ZD, Markovic SD (2015). Chelate N,O-palladium(ii) complexes: synthesis, characterization and biological activity. RSC Adv 5: 86274-86281.

Ronconi L, Sadler PJ (2007). Using coordination chemistry to design new medicines. Coord Chem Rev 251: 1633-1648.

Schmid M, Zimmermann S, Krug HF, Sures B (2007). Influence of platinum, palladium and rhodium as compared with cadmium, nickel and chromium on cell viability and oxidative stress in human bronchial epithelial cells. Environ Int 33: 385-390.

Ulukaya E, Ari F, Dimas K, Ikitimur EI, Guney E, Yilmaz VT (2011a). Anti-cancer activity of a novel palladium(II) complex on human breast cancer cells *in vitro* and *in vivo*. Eur J Med Chem 46: 4957-4963.

Ulukaya E, Ari F, Dimas K, Sarimahmut M, Guney E, Sakellaridis N, Yilmaz VT (2011b). Cell death-inducing effect of novel palladium(II) and platinum(II) complexes on non-small cell lung cancer cells in vitro. J Cancer Res Clin Oncol 137: 1425-1434.

Volarevic V, Vujic JM, Milovanovic M, Kanjevac T, Volarevic A, Trifunovic SR, Arsenijevic N (2013). Cytotoxic effects of palladium(II) and platinum(II) complexes with O,O'-dialkyl esters of (S,S)-ethylenediamine-N,N'-di-2-(4-methyl) pentanoic acid on human colon cancer cell lines. JBUON 18: 131-137.

Yoshinari A, Tazawa A, Kuwata S, Ikariya T (2012). Synthesis, structures, and reactivities of pincer-type ruthenium complexes bearing two proton-responsive pyrazole arms. Chemistry – An Asian Journal 7: 1417-1425.