Bulgarian Chemical Communications, Volume 50, Special Issue C (pp. 218 – 224) 2018

Study on the cytoprotective and antioxidant *in vitro* activity of Pr(III) complex of 5-aminoorotic acid

M. S. Kondeva-Burdina[1*], A. V. Kasabova-Angelova[1], V. P. Atanasova[2], V. J. Tzankova[1], I. P. Kostova[2]

[1]*Laboratory of drug metabolism and drug toxicity, Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University of Sofia, Sofia-1000, Dunav str. 2, Bulgaria*
[2]*Department of Chemistry, Faculty of Pharmacy, Medical University of Sofia, Sofia-1000, Dunav str. 2, Bulgaria*

Received September 26, 2017; Revised November 13, 2017

This study investigates the possible cytoprotective and antioxidant potential of Pr(III) complex of 5-aminoorotic acid (PrAOA), at a concentration of 100 μM, on sub-cellular (isolated rat liver microsomes) and cellular (isolated rat hepatocytes) level. The Pr(III) complex of 5-aminoorotic acid (PrAOA) was synthesized by reaction of the respective inorganic salt in amounts equal to ligand molar ratio of 1: 3. The newly synthesized complex was characterized by means of elemental analysis, FTIR and FTRaman spectroscopies. The effects of PrAOA were evaluated on two toxicity models: non-enzyme lipid peroxidation and *tert*-butyl hydroperoxide (*t*-BuOOH) and compared to those of 5-aminoorotic acid and quercetin. On isolated rat liver microsomes, in conditions of non-enzyme lipid peroxidation, PrAOA complex revealed good statistically significant antioxidant activity (decreasing malondialdehyde (MDA) production – marker for lipid peroxidation), closer to that of quercetin and stronger than that of 5-aminoorotic acid (AOA). On isolated rat hepatocytes, we determined the main parameters of the hepatocytes' functional and metabolic status: cell viability (measured by trypan blue exclusion), levels of lactate dehydrogenase (LDH), reduced glutathione (GSH) and MDA. In *t*-BuOOH-induced oxidative stress, PrAOA complex showed statistically significant cytoprotective and antioxidant activities, closer to those of quercetin and stronger than those of AOA. The complex prevented the loss of cell viability and GSH depletion, decreased LDH leakage and MDA production. The stronger hepatoprotective and antioxidant activity of PrAOA than that of AOA on both *in vitro* toxicity models, might be due to the presence of Pr(III) ions in the complex.

Keywords: Praseodymium(III), 5-Aminoorotic acid, Rat microsomes, Rat hepatocytes, Cytoprotection, Antioxidant

INTRODUCTION

Over the last decades lanthanide complexes have attracted much attention not only for being valuable catalysts in organic synthesis but also for their wide applications in material and biological sciences mainly due to their vary narrow emission bands, long excited-state lifetimes, large Stokes shifts, etc. About the biological activity of these complexes there is no enough information. Some authors found anti-cancer activity of lanthanide complexes. There is an information about the role of lanthanum in suppressing tumor growth as: inhibits the uptake of iron; inhibits ROS (Reactive oxygen species) formation by connecting with hydro-peroxides; masks free radicals by magnetic interaction. Lanthanide ions are of great interest to scientists. They separately, as well as coumarins, exhibit anti-tumor properties. Therefore their complexes have such activity [1-3]. Through experiments using the HL-60 (human leukocytoma) cells it has been found that complexes of Ce(III), La(III) and Nd(III) with 3,3'-ortho-pyridine methylene di(4-hydroxy coumarin) were potent cytotoxic agents, although significant differences in the IC_{50}-values were not detected. Cerium complex showed the highest activity, and neodymium the lowest one [2, 4].

Sm(III), Gd(III), and Dy(III) complexes with coumarin-3-carboxylic acid were tested for antiproliferative activity on K-562 cell line (derivatives of chronic myeloid leukemia). Samarium salt showed a lower cytotoxic activity on these cells. This was in contrast, however, with the Sm(III) complex, which had a very strong activity [5].

There are literature data about the antioxidant effect of some lanthanide complexes in blood plasma. Complexes of cerium, lanthanum and neodymium with 5-aminoorotic acid (AOA) exerted a strong antioxidant effect on the formation of radicals released in the blood plasma of Wistar rats [4].

According to this information, in this study we investigated a possible cytoprotective and antioxidant activity of Pr(III) complex of 5-aminoorotic acid (PrAOA) on sub-cellular (isolated

* To whom all correspondence should be sent:
E-mail: : magdalenakondeva@gmail.com

rat liver microsomes) and cellular (isolated rat hepatocytes) level.

MATERIAL AND METHODS

Synthesis of the of Pr(III) complex of 5-aminoorotic acid

The compounds used for preparing the solutions for the synthesis were Sigma-Aldrich products, p.a. grade: $Pr(NO_3)_3.6H_2O$ and 5-aminoorotic acid. 5-Aminoorotic acid (Fig. 1) was used as a ligand for the preparation of the metal complex.

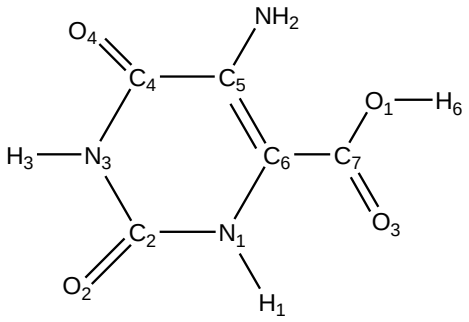

Fig. 1. The structure of the ligand 5-aminoorotic acid (AOA)

The carbon, hydrogen and nitrogen contents of the compound were determined by elemental analysis.

The solid-state infrared spectra of the ligand and its Pr(III) complex were recorded in KBr in the 4000-400 cm^{-1} frequency range on a FT-IR 113V Bruker spectrometer.

The Raman spectra of AOA and its new Pr(III) complex were recorded with a Dilor microspectrometer (Horiba-Jobin-Yvon, model LabRam) equipped with a 1800 grooves/mm holographic grating. The 514.5 nm line of an argon ion laser (Spectra Physics, model 2016) was used for the probes excitation. The spectra were collected in a backscattering geometry with a confocal Raman microscope equipped with an Olympus LMPlanFL 50× objective and with a resolution of 2 cm^{-1}. The detection of Raman signal was carried out with a Peltier-cooled CCD camera. Laser power of 100 mW was used in our measurements.

Chemicals

The chemicals used in the biological experiments were: pentobarbital sodium (Sanofi, France), HEPES (Sigma Aldrich, Germany), NaCl (Merck, Germany), KCl (Merck), D-glucose (Merck), $NaHCO_3$ (Merck), KH_2PO_4 (Scharlau Chemie SA, Spain), $CaCl_2.2H_2O$ (Merck), $MgSO_4.7H_2O$ (Fluka AG, Germany), collagenase from *Clostridium histolyticum* type IV (Sigma Aldrich), albumin, bovine serum fraction V, minimum 98 % (Sigma Aldrich), EDTA (Sigma Aldrich), 2-thiobarbituric acid (4,6-dihydroxypyrimidine-2-thiol; TBA) (Sigma Aldrich), trichloroacetic acid (TCA) (Valerus, Bulgaria), 2,2'-dinitro-5,5'-dithiodibenzoic acid (DTNB) (Merck), lactate dehydrogenase (LDH) kit (Randox, UK) and *tert*-butyl hydroperoxide (Sigma Aldrich).

Animals

Animals were purchased from the National Breeding Center, Sofia, Bulgaria. At least 7 days of acclimatization was allowed before the commencement of the study. The health was monitored regularly by a veterinary physician. The vivarium (certificate of registration of farm № 0072/01.08.2007) was inspected by the Bulgarian Drug Agency in order to check the husbandry conditions (№ A-11-1081/03.11.2011). All performed procedures were approved by the Institutional Animal Care Committee and made according Ordinance № 15/2006 for humaneness behavior to experimental animals.

Isolation of liver microsomes

Liver was perfused with 1.15 % KCl and homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH=7.4. The liver homogenate was centrifuged at 9 000 × g for 30 min at 4°C and the resulting post-mitochondrial fraction (S9) was centrifuged again at 105 000 × g for 60 min at 4°C. The microsomal pellets were re-suspended in 0.1 M potassium phosphate buffer, pH=7.4, containing 20 % glycerol. Aliquots of liver microsomes were stored at -70°C until use [6]. The content of microsomal protein was determined according to the method of Lowry and co-workers, using bovine serum albumin as a standard [7].

$FeSO_4$/ascorbic acid-induced lipid peroxidation in vitro

As a system, in which metabolic activation may not be required in the production of lipid peroxide, 20 μM $FeSO_4$ and 500 μM ascorbic acid were added directly into rat liver microsomes and incubated for 20 min at 37°C [8].

As microsomes are a sub-cellular fraction, which includes only parts of endoplasmatic reticulum, and whole cells – hepatocytes, we used different methods for measuring the MDA production.

Lipid peroxidation in microsomes

After incubation of microsomes (1 mg/ml) with the compounds, we added to the microsomes 1 ml

of 20 % (w/v) trichloroacetic acid (TCA) and 1 ml of 0.67 % 2-thiobarbituric acid (TBA). The mixture was heated at 100 °C for 20 min. The absorbance was measured at 535 nm, and the amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ [9].

Isolation and incubation of hepatocytes

An optimized i*n situ* liver perfusion using less reagents and shorter time of cell isolation was performed [10]. The method resulted in a higher amount of live and metabolically active hepatocytes.

Rats were anesthetized with sodium pentobarbital (0.2 ml/100 g). After portal catheterization, the liver was perfused with HEPES buffer (pH = 7.85) + 0.6 mM EDTA (pH = 7.85), followed by HEPES buffer (pH = 7.85) and finally HEPES buffer containing collagenase type IV (50 mg/200 ml) and 7 mM $CaCl_2$ (pH = 7.85). The liver was excised, minced into small pieces, and hepatocytes were dispersed in Krebs-Ringer-bicarbonate (KRB) buffer (pH = 7.35) + 1 % bovine serum albumin.

Cells were diluted with KRB to make a suspension of about 3×10^6 hepatocytes/ml. Incubations were carried out in flasks containing 3 ml of the cell suspension (i.e. 9×10^6 hepatocytes) and were performed in a 5 % CO_2 + 95 % O_2 atmosphere.

The rat liver microsomes and hepatocytes were incubated with a concentration of 100 μM of the complex PrAOA, 5-amino-orotic acid (AOA) and quercetin (Q) [11].

The cell viability was measured by using Trypan blue (0.05 %) as reagent [10]. Initial viability averaged 89-90 %.

Lactate dehydrogenase (LDH) release

LDH release in isolated rat hepatocytes was measured spectrophotometrically using a LDH kit [10].

Reduced glutathione (GSH) depletion

At the end of the incubation, isolated rat hepatocytes were recovered by centrifugation at 4°C, and used to measure intracellular GSH, which was assessed by measuring non-protein sulfhydryls after precipitation of proteins with trichloroacetic acid (TCA), followed by measurement of thiols in the supernatant with DTNB. The absorbance was measured at 412 nm [10].

Malondialdehyde (MDA) assay

Hepatocyte suspension (1 ml) was taken and added to 0.67 ml of 20 % (w/v) TCA. After centrifugation, 1 ml of the supernatant was added to 0.33 ml of 0.67 % (w/v) 2-thiobarbituric acid (TBA) and heated at 100°C for 30 min. The absorbance was measured at 535 nm, and the amount of TBA-reactants was calculated using a molar extinction coefficient of MDA 1.56×10^5 $M^{-1}cm^{-1}$ [10].

Statistical analysis

Statistical analysis was performed using statistical programme 'MEDCALC'. Results are expressed as mean ± SEM for 6 experiments. The significance of the data was assessed using the nonparametric Mann–Whitney test. Values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ were considered statistically significant. Three parallel samples were used.

RESULTS

The complex was synthesized by reaction of Pr(III) salt and the ligand, in amounts equal to metal: ligand molar ratio of 1: 3. The synthesis was made in different ratios (1:1, 1:2, 1:3) but in all the cases the final product was with the composition 1:3. The complex was prepared by adding an aqueous solution of Pr(III) salt to an aqueous solution of the ligand subsequently raising the pH of the mixture gradually to ca. 5.0 by adding a dilute solution of sodium hydroxide. The reaction mixture was stirred with an electromagnetic stirrer at 25 °C for one hour. At the moment of mixing of the solutions, precipitate was obtained. The precipitate was filtered (pH of the filtrate was 5.0), washed several times with water and dried in a desiccator to constant weight. The obtained complex was insoluble in water, methanol and ethanol, but well soluble in DMSO.

Reaction of Pr(III) and 5-aminoorotic acid afforded a complex which was found to be quite stable both in solid state and in solution. The new Pr(III) complex was characterized by elemental analysis. The content of the metal ion was determined after mineralization. The used spectral analyses confirmed the nature of the complex.

The data of the elemental analysis of the Pr(III) complex served as a basis for the determination of its empirical formula and the results are presented below. The found elemental analysis of Pr(III) complex of AOA ($Pr(AOA)_3.H_2O$) is shown as % calculated/found: C= 26,90/26,64; H= 2,09/2,29; N=18,83/19,00; H_2O= 2,69/2,25; Pr= 21,07/20,76, where HAOA= $C_5N_3O_4H_5$ and AOA^-= $C_5N_3O_4H_4^-$.

In our previous work the geometry of 5-aminoorotic acid was computed and optimized with the Gaussian 03 program employing the B3PW91 and B3LYP methods with the 6-311++G** and

LANL2DZ basis sets [12]. In the present study the binding mode of the AOA ligand to Pr(III) ions was elucidated by recording the IR and Raman spectra.

In the well-defined high-frequency field present in the IR and Raman spectra, extreme intensity changes were observed in going from the acid to the complex. In the spectral region 3500–2000 cm^{-1} the O–H, N–H, and C–H stretches give rise to intense IR bands. The involvement of these groups in hydrogen bonds produces a relevant band broadening in the IR and Raman spectra.

The double bond stretching vibrations ν(C=O) and ν(C=C) are the internal coordinates that dominate in the modes with fundamentals in the 1800–1600 cm^{-1} spectral range. One very strong band can be observed at 1691 cm^{-1} in the IR spectrum of the ligand assigned to the symmetrical stretching mode of C=O of the heterocyclic molecule. Opposite to the IR spectra, in this region of the Raman spectra only a medium band at 1699 cm^{-1} for the free ligand was observed. These bands (broad and relatively strong in the IR spectrum) shifted in the spectra of the title complex. The same shifts were observed for the strong IR band at 1667 cm^{-1} tentatively assigned to the ν(C=O) mode of the carboxylic group and for the experimental Raman band at 1341 cm^{-1} assigned to the stretching ν(C–O) mode as a medium signal from the spectra of the free ligand. It has to be mentioned that strong H-bonds are expected through the carboxylic group. Different stretches of the uracil ring contributed to the bands in the 1600–900 cm^{-1} region slightly shifted in the spectra of the title complex.

The metal affects the carboxylate anion, as well as the ring structure. The spectra in the frequency region below 600 cm^{-1} are particularly interesting, since they provide information about the metal-ligand vibrations. The new bands in the 600–500 cm^{-1} region present only in the IR spectrum of the complex can be due to the Pr-O interactions. The Raman spectra are particularly useful in studying the metal-oxygen stretching vibrations, since these vibrations give rise to medium intensity bands in Raman, but are weak in the infrared spectra. The observed bands and their assignments are in accordance with the literature data for similar coordination compounds [12].

Effect of PrAOA on MDA activity in isolated microsomes

Microsomes incubation with Fe^{2+}/AA, resulted in statistically significant increase of the amount of malondialdehyde (MDA) with 191 % *vs* control (non-treated microsomes).

In the non-enzyme-induced lipid peroxidation model, pre-treatment with PrAOA and AOA (at concentration 100 μM) significantly reduced lipid damage by 71 % and 44 % respectively, as compared to the toxic agent (Fe^{2+}/AA) (Fig. 2). At the same concentration quercetin, used as a control, lowered MDA formation by 73 %.

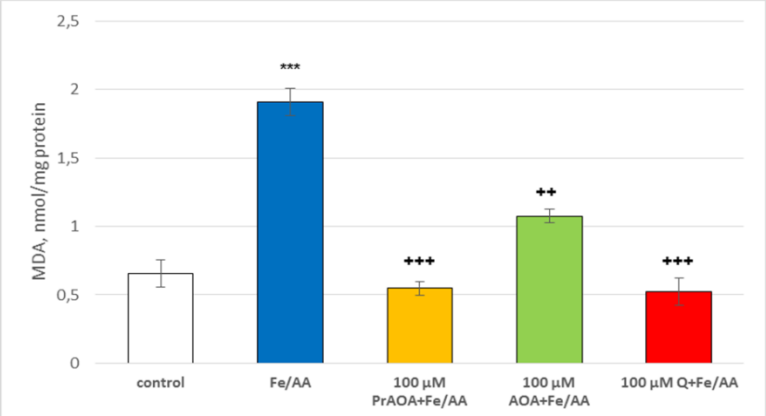

Fig. 2. Effects of PrAOA, AOA and quercetin on MDA production in conditions of non-enzyme lipid peroxidation on isolated rat liver microsomes. *** P < 0.001 *vs* control (non-treated microsomes). ++ P < 0.01; +++ P < 0.001 *vs* toxic agent (Fe^{2+}/AA)

Effects of PrAOA on parameters, characterizing the functional-metabolic status of isolated rat hepatocytes

One of the most useful models of oxidative stress is *t*-BuOOH (at concentration 75 μM) [13]. Administered alone, *t*-BuOOH leads to significant reduction of cell viability by 77 %, increased LDH activity by 150 %, depletion of cell GSH by 65 % and increased lipid peroxidation by 210 %,

compared to the control (non-treated hepatocytes) (Figs. 3 and 4).

In this model of oxidative stress, pre-treatment with PrAOA and AOA (at concentration 100 μM) significantly preserved cell viability by 183 % and 117 %, respectively, as compared to the toxic agent (*t*-BuOOH) (Fig. 3). At the same concentration quercetin, used as a control, preserved cell viability by 273 %.

Increased LDH leakage is a sign for cellular damage. Pre-treatment with PrAOA and AOA significantly decreased LDH leakage by 100 % and 70 % respectively, as compared to the toxic agent (*t*-BuOOH) (Fig. 3). At the same concentration quercetin, used as a control, decreased LDH leakage by 150 %.

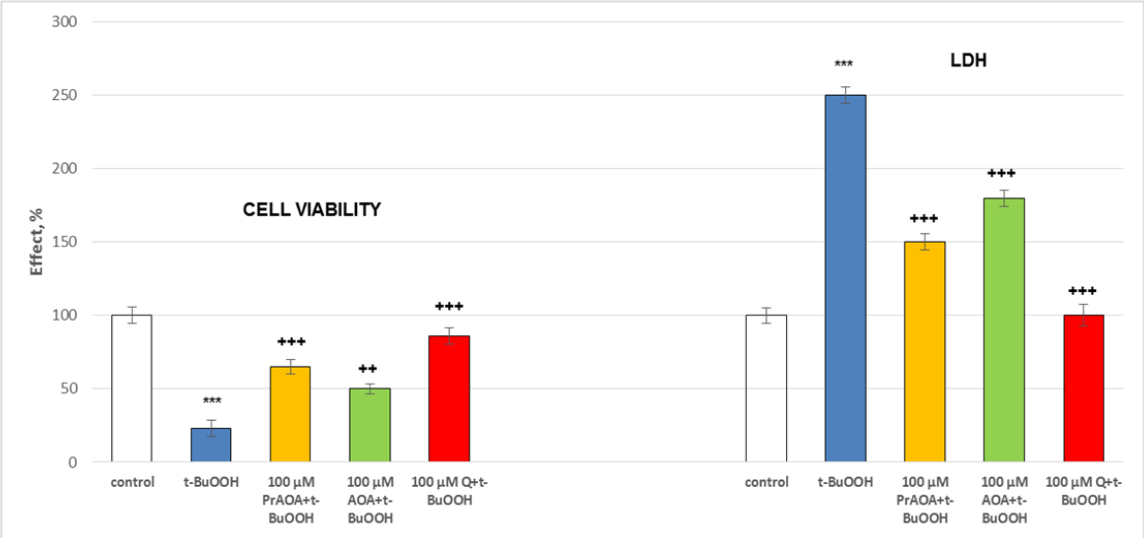

Fig. 3. Effects of PrAOA, AOA and quercetin on cell viability and LDH leakage in conditions of *t*-BuOOH-induced oxidative stress on isolated rat hepatocytes. *** P < 0.001 *vs* control (non-treated hepatocytes) ++ P < 0.01; +++ P < 0.001 *vs* toxic agent (*t*-BuOOH).

Reduced glutathione (GSH) is one of the main hepatic protectors. It is a nucleophile, which binds electrophiles, like ROS. Decreased level of GSH is a sign for cellular damage. Pre-treatment with PrAOA and AOA significantly preserved GSH level by 60 % and 37 % respectively, as compared to the toxic agent (*t*-BuOOH) (Fig. 4). At the same concentration quercetin, used as a control, preserved GSH level by 137 %.

The main marker for lipid peroxidation is the high production of malondialdehyde (MDA). Pre-treatment with PrAOA and AOA significantly decreased MDA production by 60 % and 30 % respectively, as compared to the toxic agent (*t*-BuOOH) (Fig. 4). At the same concentration quercetin, used as a control, decreased MDA production by 130 %.

DISCUSSION

In experimental toxicology, the *in vitro* systems are widely used for the investigation of the xenobiotics biotransformation, and for revealing the possible mechanisms of toxic stress and its prevention.

Some of the most suitable sub-cellular *in vitro* systems for investigation of drug metabolism are isolated microsomes. Normally in the cells, the microsomes do not exist. They are prepared artificially by differential centrifugation, and represent fragments from endoplasmic reticulum. Microsomes preserve the enzyme activity, mostly cytochrome P450 enzymes and can also be used as a model of lipid membrane in experiments, related to the processes of lipid peroxidation [14].

Here, we show that the complex PrAOA revealed a statistically significant antioxidant effect, stronger than that of AOA and similar to that of the classical hepatoprotector quercetin, in non-enzyme-induced lipid peroxidation at isolated microsomes. Our results demonstrated that MDA level in the samples treated with PrAOA, was markedly decreased.

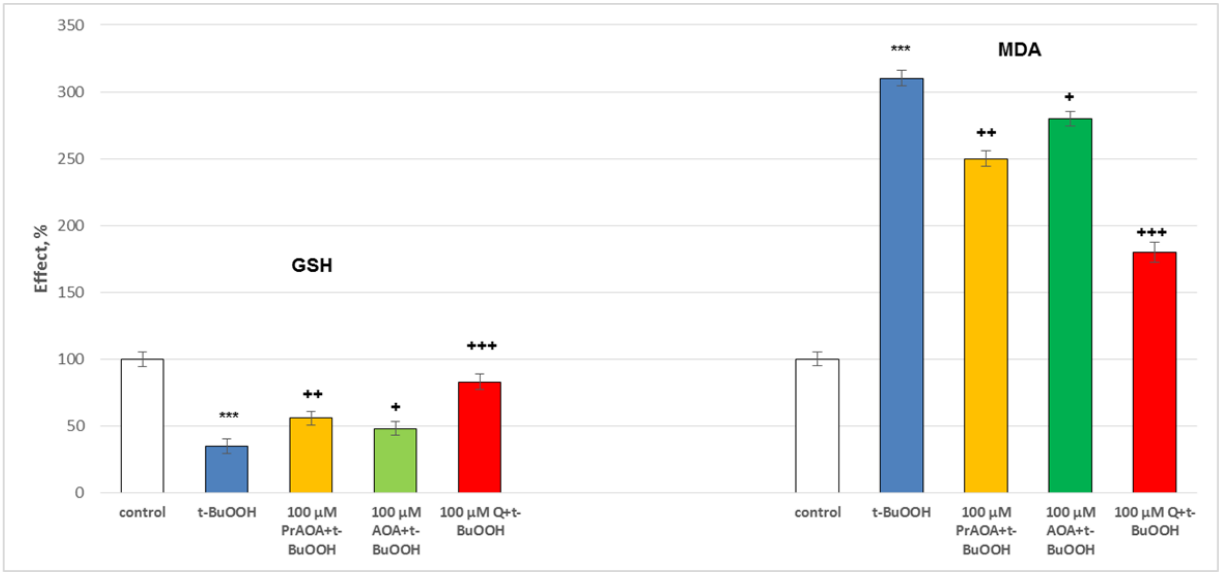

Fig. 4. Effects of PrAOA, AOA and quercetin on GSH level and MDA production in conditions of *t*-BuOOH-induced oxidative stress on isolated rat hepatocytes. *** P < 0.001 vs control (non-treated hepatocytes) + P < 0.05; ++ P < 0.01; +++ P < 0.001 vs toxic agent (*t*-BuOOH)

Isolated liver cells are a convenient model system for evaluation of the cytoprotective effects of some promising biologically active compounds, both newly synthesized and derived from plants.

One of the classical models of oxidative stress is *tert*-butyl hydroperoxide (*t*-BuOOH). Two mechanisms for *t*-BuOOH action were proposed: depletion of GSH cellular stores and oxidation of functionally important SH groups on mitochondrial enzymes, and/or changes of mitochondrial membrane integrity induced by peroxidation of membrane lipids [13].

The results from the present study showed that in the model of *t*-BuOOH-induced oxidative stress, PrAOA had statistically significant cytoprotective and antioxidant activities, stronger than those of AOA and similar to those of quercetin.

The results from this study correlate with previous results about the antioxidant effect of some lanthanide complexes in blood plasma. Complexes of cerium, lanthanum and neodymium with 5-aminoorotic acid exerted a strong antioxidant effect on the formation of radicals released in the blood plasma of Wistar rats [4].

Lanthanides are considered of high potential because of their inherent antioxidant properties. There are other literature data, which support that in conditions of *tert*-butyl hydroperoxide-induced oxidative stress, Ln(III) lost reactivity of produced peroxides, when they were bound to membrane. The lanthanide inhibiting ROS involves strong oxyphilicity inherent to lanthanides, because of the availability of oxygen sites on these free radicals, making them excellent targets for Ln(III) coordination (attack). This causes lanthanide to play the role of scavenger of reactive oxygen species, therefore presenting good potential for lanthanide as a future drug for a number of degenerative diseases due to ROS. Ln(III) very easily interacts with either free radicals or peroxides but is not transformed as radicals [15].

We suggest that these antioxidant effects of PrAOA might be due to possible mechanism as those of other Ln(III) complexes.

CONCLUSION

The present study provides novel and important data on the *in vitro* cytoprotective and antioxidant activity of the newly synthesised Pr(III) complex of 5-aminoorotic acid (PrAOA) on different toxicity models on sub-cellular (isolated rat liver microsomes) and cellular level (isolated rat hepatocytes). The complex was characterized by elemental and vibrational spectral analyses, including IR and Raman spectra. The complex PrAOA revealed statistically significant antioxidant and cytoprotective effects, stronger than those of 5-aminoorotic acid and similar to those of classical hepatoprotector quercetin.

Acknowledgements: *The study was carried out with the financial support by the Medical University of Sofia (Council of Medical Science, Project № 463, Contract 46/2016)*

REFERENCES

1. K.F. Devienne, A.F. Carlgaro-Helena, D.J. Dorta, I.M. Prado, M.S. Raddi, W. Vilegas, S.A. Uyemura, A.C. Santos, C. Curti, *Phytochemistry*, **68**, 1075 (2007).
2. B. Thati, A. Noble A, B.S. Creaven, M. Walsh, M. McCann, K. Kavanagh K, M. Devereux, D.A. Egan, *Cancer Lett.*, **248**, 321 (2007).
3. Y. Yu, L. Wang, R.L. Walzem, E.G. Miller, L.M. Pike, B.S. Patil, *J. Agric. Food. Chem.*, **53**, 2009 (2005).
4. I. Kostova, M. Traykova, V. K. Rastogi, *Med. Chem.*, **4**, 371 (2008).
5. I. Kostova, G. Momekov, P. Stancheva, *Met. Based Drugs*, 15925 (2007).
6. R.L. Simeonova, V.B. Vitcheva, M.S. Kondeva-Burdina, I.N. Krasteva, S.D. Nikolov, M.K. Mitcheva, *Phytomedicine*, **17**, 346 (2010).
7. O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randal, *J. Biol. Chem.*, **193,** 265 (1951).
8. H.J. Kim, Y.J. Chun, J.D. Park, S.I. Kim, J.K. Roh, T.C. Jeong, *Planta Med.*, **63**, 415 (1997).
9. E.D. Wills, *Biochem. J.*, **99**, 667 (1966).
10. M. Mitcheva, M. Kondeva, V. Vitcheva, P. Nedialkov, G. Kitanov, *Redox Rep.*, **11**, 1 (2006).
11. K.Y. Oh, V.H. Roberts, M.C. Schabel, K.L. Grove, M. Woods, A.E. Frians, *Radiology*, **276**, 110 (2015).
12. I. Kostova, N. Peica, W. Kiefer, *Chem. Phys.*, **327,** 494 (2006).
13. V. O'Donell, M.J. Burkit, *Biochem. J.*, **304**, 707 (1994).
14. I. Zaidi, R. Agarwal, G. Eichler, B.D. Rihter, M.E. Kenney, H. Mukhtar, *Photochem. Photobiol.*, **58**, 204 (1993).
15. S.N. Misra, M.A. Gagnani, I.M. Devi, R.S. Shukla, *Bioinorg. Chem. Allp.*, **2**, 155 (2004).

ИЗСЛЕДВАНЕ НА ЦИТОПРОТЕКТИВНАТА И АНТИОКСИДАНТНА *in vitro* АКТИВНОСТ НА PR(III) КОМПЛЕКС С 5-АМИНООРОТОВА КИСЕЛИНА

М. С. Кондева-Бурдина[1*], А. В. Касабова-Ангелова[1], В.П. Атанасова[2], В.Й. Цанкова[1], И.П. Костова[2]

[1] Лаборатория по метаболизъм и токсичност на лекарствата, Департамент по фармакология, фармакотерапия и токсикология, Факултет по фармация, Медицински университет, ул. Дунав 2, София 1000, България

[2] Департамент по химия, Факултет по фармация, Медицински университет, ул. Дунав 2, София 1000, България

Постъпила на 26 септември, 2017 г.; коригирана на 13 ноември, 2017 г.

(Резюме)

В настоящата работа е изследван възможният цитопротективен и антиоксидантен потенциал на комплекса на Pr(III) с 5-аминооротовата киселина (PrAOA) с концентрация100 μM, на субклетъчно (изолирани микрозоми от черен дроб на плъх) и клетъчно (изолирани хепатоцити от плъх) ниво. Комплексът на Pr(III) с 5-аминооротовата киселина (PrAOA) е синтезиран чрез реакция на съответната неорганична сол в количество, отговарящо на моларно съотношение с лиганда от 1: 3. Новосинтезираният комплекс е характеризиран чрез елементен анализ, FTIR и FTRaman спектроскопия. Влиянието на PrAOA е изследвано с помощта на два модела токсичност: неензимна липидна пероксидация и трет.-бутилов хидропероксид (*t*-BuOOH) и е сравнено с тези на 5-аминооротовата киселина и кверцетина. Върху изолирани микрозоми на черен дроб от плъх при условия на неензимна липидна пероксидация, PrAOA комплексът проявява добра статистически значима антиоксидантна активност (намалявайки продукцията на малонов дианхидрид (MDA)– маркер за липидна пероксидация), близка до тази на кверцетина и по-силна от тази на 5-аминооротовата киселина (AOA). Върху изолирани хепатоцити са определени основните параметри на функционалния и метаболитния статус на хепатоцитите: клетъчна жизнеспособност (измерена чрез ексклузия на трипаново синьо), нивата на лактат дехидрогеназа (LDH), редуциран глутатион (GSH) и MDA. При *t*-BuOOH-индуциран оксидативен стрес, PrAOA комплексът проявява статистически значима цитопротективна и антиоксидантна активност, близка до тази на кверцетина и по-силна от тази на AOA. Комплексът предотвратява загубата на клетъчна жизнеспособност и изчерпването на GSH, намалява загубата на LDH и производството на MDA. По-силната хепатопротективна и антиоксидантна активност на PrAOA в двата *in vitro* модела на токсичност от тази на AOA би могла да се дължи на присъствието на Pr(III) йони в комплекса.