PharmacologyOnLine

Archives • 2021 • vol. 3 • 1966-1972

THE STUDY OF THE MECHANISM AND POSSIBLE CORRECTION OF NANOCHROMIUM CITRATE TOXIC ACTION ADMINISTERING THIOTRIAZOLINE AS AN ANTIOXIDANT DRUG

[1]Sadohurska Kateryna*, [1]Greshko Iuliia, [2]Voytkevich Yulia, [3]Sytnyk Inna
[1]Bukovinian State Medical University, Chernivtsi, Ukraine
[2]"Dobrobut" Clinic, Therapeutic Department, Kyiv, Ukraine
[3] Bogomolets National Medical University, Kiev, Ukraine

*sadogurska.katya@ukr.net

Abstract

Thiotriazoline effect on nanochromium citrate (NCC) toxicity and the state of pro- and antioxidant homeostasis with underlying single administration of sub-lethal and lethal NCC doses were studied in the acute experiment on laboratory rats. Thiotriazoline (100 mg/kg, 7 days) was found to reduce the signs of intoxication, prolong life and increase survival of animals. Intoxication of animals with high doses of NCC (DL_{100}, DL_{50}, DL_0) is accompanied by intensification of the processes of free radical lipid oxidation and protein oxidative modification in the body, decrease of catalase activity and the content of reduced glutathione in erythrocytes, the content of free SH-groups and general antioxidant activity in the blood plasma. A single administration of Thiotriazoline prevents development of oxidant-antioxidant homeostasis disorders in the animal bodies with underlying minimum toxic dose of NCC, but in case of lethal doses a single administration of Thiotriazoline is of little effect.

Keywords: *nanochromium citrate, toxicity, pro-, antioxidant homeostasis, Thiotriazoline*

Introduction

Due to nanotechnologies the current stage of scientific development is supplied with new materials possessing unique properties. Chemical substances in a nanometer range acquire totally new properties, which make it possible to use them wider [1, 2]. Meanwhile, development of nanotechnologies and obtaining new nanomaterials promote scientists to investigate their safety with the purpose to avoid possible unfavorable consequences both for human health and environment.

Ukrainian State Scientific-Research Institute of Nanobiotechnologies and Resource-Saving received organic chromium compound – nanochromium citrate (NCC) by means of electric pulse aquananotechnology [3]. This compound of chromium as a vital trace element [4] aroused an interest to learning its biological activity and prospects of its use in biology and medicine. Supplement of chromium nanoparticles to the main diet of animals is found to improve the appetite and enhance an average daily mass increase [5]. The current scientific literature contains experimental researches concerning a positive effect of NCC on the metabolic processes in the bodies of domestic animals (pigs, rabbits) and its possible use as a food additive in the vet practice [6]. Our previous studies found that NCC toxicity does not depend on the dose given only but the ways of administration into the organism as well. Thus, in case of enteral use according to K.K. Sidorov's classification (1973) NCC can be considered as a "Moderately toxic compound", but in case of intravenous introduction or into the peritoneum it becomes a "highly toxic compound" [7].

One of the ways to increase the safety of NCC could be indication of correcting substances producing a metabolic action, and a Ukrainian pharmacological agent Thiotriazoline (morpholin 3-methyl-1,2,4-triazol-5-thioacetic acid) in particular. From the chemical point of view Thiotriazoline pharmacophore is triazol nucleus possessing non-localized p-electron cloud with a high reactive ability. Sulfur atom in the molecule possesses a certain excessive electron density due to unequal effect of heterocyclic and carbonyl groups on it. Due to such chemical structure Thiotriazoline possesses a high biological activity and low toxicity [8]. Thiotriazoline was used in medical practice at the end of 90-s of the last century as a metabolic and trophic agent with an expressed antioxidant activity and polytrophic spectrum of therapeutic action. It has hepatoprotective, cardioprotective, nephroprotective, immunomodulating and other properties [9]. The ability of Thiotriazoline to produce simultaneous effect on different organs and systems, its safety in use, convenient pharmacological forms are its advantages in comparison with other known metabolic and trophic agents. Moreover, Thiotriazoline manifests anti-toxic action with heavy metals intoxication. Though, Thiotriazoline protective activity concerning negative effects of NCC has not been studied yet.

Objective of the study was investigation of a correcting Thiotriazoline effect on general toxicity and parameters of biochemical detoxification in case of toxic doses of nanochromium citrate.

Methods

The study was carried out on mature laboratory rats with the body weight of 150 - 200 g. The animals were divided into 2 groups. In the first group (42 animals) Thiotriazoline effect on acute NCC toxicity (Ltd "Nanomaterials and Nanotechnology", 200 mg/L) was studied when it was introduced in sub-lethal and lethal doses: 3 mg/kg (DL_0), 4.5 mg/kg (DL_{50}) and 5 mg/kg (DL_{100}) [7]. 2 hours after a single introduction of the compound into the peritoneum to correct toxicity with a therapeutic-preventive purpose Thiotriazoline (SC "Halychpharm") during 7 days in the dose of 100 mg/kg [10]. Water for injection was administered in the similar volume to the control animals (intact control). Survival results of the experimental animals were compared with those intoxicated with NCC (not treated) and with the intact control. The animals were observed during 14 days after NCC introduction. The external view, behavior, rhythm and respiratory rate, attitude to food, time of occurrence and signs of intoxication, its course, and the time of death of the animals were observed. In the following group of experiments (42 animals) the parameters of pro- and antioxidant balance in the body of rats were studied in the ground of a single administration of toxic doses of NCC (3 mg/kg, 4.5 mg/kg, 5 mg/kg) and Thiotriazoline (100 mg/kg). Biochemical examinations were made the following day. The blood and liver of the animals were biological substrates for these examinations. The content of Malone aldehyde (MA) by the reaction with thiobarbituric acid [11], the content of reduced glutathione (GSH) by means of titer metric method and catalase activity were measured in the blood erythrocytes [12]. Total antioxidant capacity (TAC), the content of oxidative-modified proteins (OMP) [13] and free HS-groups [14] were determined in the blood plasma. The liver homogenates (5%) of animals were prepared cold on 50 mM tris-HCl-buffer (pH=7.4). The content of MA, GSH, OMP and catalase activity were determined in the post-nuclear supernatants of homogenates.

All the studies were carried out in accordance with the criteria outlined in the European Union Directive

2010/63/EU "On the protection of animals used for scientific purposes".

Statistical processing of the obtained data was performed using the SPSS Statistics 17.0 software. All data are represented as a mean ± standard error of the mean (M±m). Estimation of the differences between the samples was conducted using a parametric Student's t-test and a nonparametric Mann-Whitney U test. The minimum significance level was $p<0.05$.

Results

In the acute experiment after a single introduction of NCC in toxic doses the animals presented inhibited motor activity, difficult breathing, and periodical tonic spasms. In the ground of general inhibition the animals died on the 2-3rd days. Thiotriazoline administration with a therapeutic purpose in the ground of sub-lethal and lethal doses of NCC decreased considerably general signs of intoxication, made the life of animals longer. The lethal outcome occurred later – on the 6-8th day. After NCC introduction in the dose of 4.5 mg/kg (DL_{50}) a half of the animals died, and survival rate of the animals treated with Thiotriazoline increased (see Table 1). After administration of an absolutely lethal dose of NCC (5 mg/kg) survival rate of the animals treated was 83%. The facts obtained are indicative of Thiotriazoline antitoxic action. The motor activity and food intake were restored in the survived animals during the first day.

In the following series of experiments in order to specify possible mechanisms of a correcting detoxification Thiotriazoline action the state of pro- and antioxidant balance was studied in the ground of toxic doses of NCC (see Tables 2, 3). A considerable oxidant-antioxidant imbalance was found in the bodies of animals with the action of toxic doses of NCC. The content of the products of free radical lipid and biopolymer oxidation increased in the blood and liver of the animals non-treated. With the increase of NCC dose (3 mg/kg, 4.5 mg/kg, 5 mg/kg) MA content in the blood erythrocytes of the rats in comparison with the control animals 18%, 22%, 27% increased (see Table 2), and in the liver – 19%, 25%, 35% respectively (see Table 3). With the increase of NCC dose the content of OMP in the blood serum 19%, 26%, 37% increased as well, and in the liver – 34% - 36%, which is indicative of the activation of the pro-oxidant system in the bodies of intoxicated animals. Considering the parameters that characterize the system of antioxidant protection with introduction of NCC in the above doses, it should be noted that in all the animals that received high doses of the compound the total antioxidant capacity of the blood plasma 27%, 30%, 47% decreased respectively, and the content of SH-groups – 11%, 14%, 33% decreased (see Table 2). The content of GSH as one of the major endogenous antioxidants decreased as well in the blood and liver of rats (in the erythrocytes of the blood - 17%-45%, and in the liver – 13%-35%). High doses of NCC resulted in reduced activity of catalase, as one of the key enzymes of the antioxidant protection, both in the blood and in the liver (see Tables 2, 3).

Discussion

The current scientific literature contains disputable information concerning the effect of chromium compounds on the antioxidant protection systems. Chromium cations (III) are able to act in the body as antioxidants, and in high doses as pro-oxidants, which correlates with the data obtained in our research.

Introduction of Thiotriazoline in the ground of intoxication with high doses of NCC prevents the advance of disorders in the oxidant-antioxidant balance in the bodies of intoxicated rats. At the same time, Thiotriazoline action was found to be most effective when NCC was introduced in minimum toxic doses. TAC in the blood plasma increased reliably in the rats of this group in comparison with the animals not treated, and the content of SH-groups did not differ from the parameters of the intact animals (see Table 2). Since a single Thiotriazoline introduction in the ground of lethal doses of NCC appeared to be little effective, its long (course) administration could be more reasonable with a correcting purpose which is confirmed by the results obtained in our studies on the reduction of a compound toxic action and increase of the survival rate of animal with a repeated introduction of Thiotriazoline in the ground of toxic doses of NCC (see Table 1). Scientific literary data indicate that Thiotriazoline as a metabolic and trophic therapeutic agent inhibits formation of oxygen active forms, prevents oxidative modification of protein structures, can compete with SH-groups of protein molecules for superoxide anion-radical, promotes normalization of catalase and glutathione peroxidase activity, and keeps reduced glutathione reserves. In general, a positive correcting effect of Thiotriazoline concerning decrease of NCC toxicity might be caused by not only its ability to restore the oxidant-antioxidant balance disturbed under conditions of intoxication, but its polytrophic action of the body described in a number of published works [8, 9].

Conclusion. A long (7 days) therapeutic-preventive introduction of Thiotriazoline (100 mg/kg) in the acute experiment on rats in the ground of toxic doses of nanochromium citrate (DL_0, DL_{50}, DL_{100}) reduces the signs of intoxication and lethal outcome of animals. Intoxication of animals with high doses of nanochromium citrate is accompanied by the intensification of the processes of free radical lipid oxidation and protein

oxidative modification in the body, reduced catalase activity, the total antioxidant capacity of the blood plasma, the content of free SH-groups and reduced glutathione. Single Thiotriazoline introduction prevents development of oxidant-antioxidant homeostasis disorders in the body of animals in the ground of minimum toxic doses of nanochromium citrate, and in the ground of lethal doses single Thiotriazoline introduction is little effective.

References

1. Chekman, I.S., Ulberg, Z.N., Malanchuk, V.O., Gorchakova, N.O., Zupanets, I.A. (2012). Nanonauka, nanobiologiya, nanofarmaciya [Nanoscience, nanobiology, nanopharmacy]. Kyiv: Ukraine.

2. Bandas, I.A., Krynytska, I.Ya., Kulitska, M.I., Korda, M. M. (2015). Nanoparticles: importance today, classification, use in medicine, toxicity. Medical and clinical chemistry, 17(3), 123-128.

3. Kosinov, M.V., Kaplunenko, V.H. (2008). Sposib otrymannya akvakhelativ nanometaliv «Eroziyno-vybukhova nanotekhnolohiya otrymannya akvakhelativ nanometaliv» [Method of obtaining nanometals aqua chelates "Erosion-explosive nanotechnology of obtaining nanometals aqua chelates"] (Ukraine Patent identifier № 29856), 2008.

4. Cefalu, W.T., Hu, F.B. (2004). Role of chromium in human health and in diabetes. Diabetes Care, 27(11), 2741-2751.

5. Zha, L., Xu, Z., Wang, M., Gu, L. (2007). Effects of chromium nanoparticle dosage on growth, body composition, serum hormones and tissue chromium in Sprague-Dawley rats. J Zhejiang Univ Sci B, 8(5), 323-330.

6. Iskra, R.Ya., Svarchevska, O.Z., Maksymovych, I.Ya. (2012). Glutathione antiperoxid system in blood and tissues of rats for the action of nanocryane citrate. Bulletin of problems of biology and medicine; 2(93), 32 – 35.

7. Sadohurska, K.V. (2015). Study of acute toxicity of nanochrome citrate by different routes of administration. Pharmaceutical Journal, 4(36), 56-59.

8. Bibik, E.Yu., Fomina, K.A., Jchak, M.V. (2009). Thiotriazoline is a potential drug with detoxifying activity. Ukrainian Medical Almanac, 12(1), 213-217.

9. Kopchuk, T.G. (2014). Pharmacological effects of thiotriazoline. Modern scientific bulletin, 10(206), 66-71.

10. Gerush, O.V., Kosuba, R.B., Pinyazhko O.R. (2003). Renal`ni efekty` tiotry`azolinu: metody`chni rekomendaciyi [Renal effects of thiotriazoline: guidelines]. Kyiv: Ukraine.

11. Steel, I.D., Garishvili, T.G. (1977) Metody` opredeleniya malonovogo dial`degida s pomoshh`yu tiobarbiturovoj kisloty` [Methods for the determination of malonic dialdehyde using thiobarbituric acid]. M.: Medicine.

12. Korolyuk, M.A., Ivanova, M.I., Mayorova, I.G. (1998). Method for determination of catalase activity, 1, 16-18.

13. Meshchishen, I.F. (1998). Method for determination of oxidatively modified plasma proteins (serum). Bukovynian Medical Bulletin, 2(1), 156-158.

14. Verevkina, I.V., Tochilkin, A.I., Popova, N.A. (1977). Kolorimetricheskij metod opredeleniya HS-grupp i S-S-svyazej v belkakh pri pomoshhi 5,5'-ditiobis(2-nitrobenzojnoj) kisloty`) [Colorimetric method for determination of HS-groups and S-S-bonds in proteins using 5,5'-dithiobis (2-nitrobenzoic acid)]. M.: Medicine.

Table 1. Thiotriazoline effect (100 mg/kg) on the survival of rats in the ground of toxic and lethal doses of nanochromium citrate (NCC), n=6

Terms of the experiment	NCC (non-treated animals)	NCC + Thiotriazioline (treated animals)
	survived / died	survived / died
Intact control	-	-
NCC, 3 mg/kg (DL_0)	6 / 0	6 / 0
NCC, 4.5 mg/kg (DL_{50})	3 / 3	5 / 1
NCC, 5 mg/kg (DL_{100})	0 / 6	5 / 1

n – the number of animals in each series.

Table 2. Nanochromium citrate effect on the parameters of the oxidant-antioxidant homeostasis in the blood of rats (M ± m; n = 6)

Terms of the experiment / Parameters	Control	Nanochromium citrate			Nanochromium citrate + Thiotriazioline		
		3 mg/kg	4.5 mg/kg	5 mg/kg	3 mg/kg	4.5 mg/kg	5 mg/kg
Free SH-groups, mcmol/ml	0.55±0.02	0.49±0.02	0.47±0.02	0.37±0.02*	0.54±0.02	0.40±0.02*	0.40±0.02*
OMP, o.o.g/ml	1.13±0.09	1.40±0.07	1.52±0.02*	1.80±0.05*	1.32±0.08	1.42±0.09	1.44±0.06#
MA, mcmol/L	12.67±0.49	15.44±0.17	16.25±0.23*	17.47±0.61*	14.03±0.33	14.70±0.18*, #	17.51±0.18*
GSH, mcmol/ml	1.72±0.04	1.43±0.04*	1.16±0.03*	0.95±0.05*	1.48±0.02*	1.39±0.04*	1.09±0.05*
Catalase, mcmol/min• L	19.8±0.72	18.01±0.37	16.15±0.27*	17.26±0.46	18.57±0.71	17.22±0.28	17.70±0.39
TAC, %	84.1±7.2	61.3±4.1*	58.5±5.6*	44.8±4.9*	72.2±5.4 #	65.3±5.2*	58.7±4.1*

* difference of the parameters is reliable (p ≤ 0.01) in comparison with the control animals;

difference of the parameters is reliable (p ≤ 0.01) in comparison with non-treated animals.

Table 3. Nanochromium citrate effect on the parameters of the oxidant-antioxidant homeostasis in the liver of rats

Terms of the experiment / Parameters	Control	Nanochromium citrate			Nanochromium citrate + Thiotriazoline		
		3 mg/kg	4.5 mg/kg	5 mg/kg	3 mg/kg	4.5 mg/kg	5 mg/kg
MA, mcmol/g	33.86±1.35	41.64±1.25*	45.37±0.37*	51.67±0.29*	37.75±0.74	45.70±1.84*	45.38±0.66*,#
OMP, o.o.g/g	17.02±0.85	25.85±1.07*	26.94±0.57*	26.39±0.42*	20.73±0.44#	22.74±0.79#	24.13±1.35
Catalase, mcmol/min• mg	19.48±0.59	14.36±0.07*	15.49±0.27*	11.93±0.36*	17.62±0.87#	15.07±0,85*	13.82±0.53*
GSH, mcmol/g	7.42±0.27	5.62±0.11*	6.29±0.18*	4.87±0.30*	6.67±0.33	6.46±0.13*	6.05±0.38

* difference of the parameters is reliable ($p \leq 0.01$) in comparison with the control animals;

difference of the parameters is reliable ($p \leq 0.01$) in comparison with non-treated animals.