The Effect of the Serum Amino Acid Levels Thiosemicarbazone Derivatives and its Metal Complexes on Rats

Mustafa Karatepe[1], Dilara Kaman[2]

[1] Department of Chemistry, Firat University, Elazig, Turkey

[2] Department of Biochemistry, Faculty of Medicine, Firat University, Elazig, Turkey

Abstract

Advers biological activities of Thiosemicarbazone (TSC) and Schiff base (SB) derivatives have been widely studied in rats and in other animal species using different doses, times and routes of administration. To date, no attempt has been made to study alterations occurring in the amino acid profile in the effects of the thiosemicarbazone derivative and its metal complexes on the rats. At this study, the rats were injected subcutaneously with a new thiosemicarbazone and its LH-Zn and LH-Cu complexes (25 mg/kg body weight) and then sacrified after 15th days. The aim of this study was to determine the effect of new compounds on the serum amino acid levels in rats. In the comparision done among groups, it was observed that serum amino acid concentrations were statistically changed ($p < 0.05$).

Key Words: Thiosemicarbazone, metal complex, amino acid

(Rec.Date: *Dec 18, 2012* - Accept Date: *Jan 24, 2013*)

Correspondence Author: Mustafa Karatepe, Department of Chemistry, Firat University, 23169, Elazig, Turkey
E-mail: mkaratepe@firat.edu.tr

Introduction

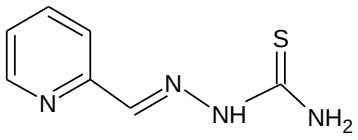
Thiosemicarbazone (TSC) and Schiff base (SB) derivatives have aroused considerable interest in chemistry and biology due to their antibacterial, antimalarial, antineoplastic and antiviral activities [1-3]. These compounds represent some of the most potent known inhibitors of ribonucleoside diphosphate reductase. The reductive conversion of ribonucleotides to their deoxyribonucleotide counterparts is a particularly critical step in the synthesis of DNA [4-6]. In addition, it is well known that some cyclobutane derivatives with other functional groups exhibit antiviral and antineoplastic activities [7,8] and liquid crystal properties [9].

5-Aminoisoquinoline-1-carboxaldehyde thiosemicarbazone (AIQ-1) that is one of the members of the TSC derivatives was considered as a second-generation drug of this class for trial against human cancer [10].

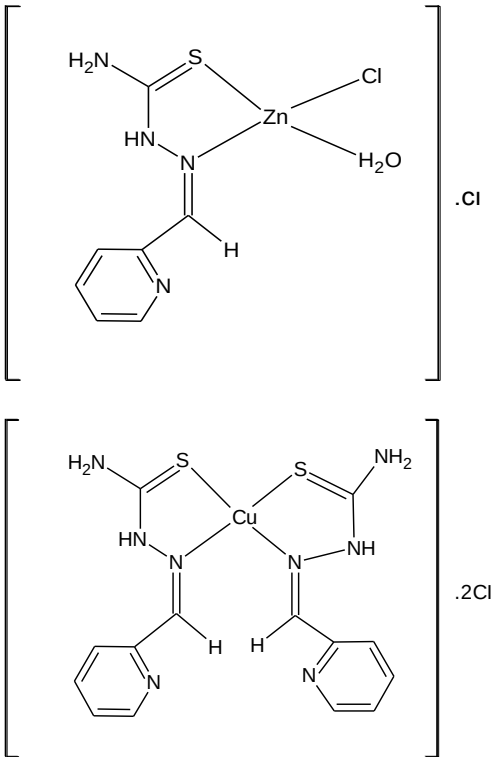
It has been found that metal complexes of TSCs were increased their biological activity [11]. TSCs usually react as ligands with metal cations by bonding through the sulfur and the azomethinic nitrogen atoms [12]. The implications of metal compounds such as palladium, cadmium and mercury in tumor growth inhibition have been the subject of several studies [13,14]. It has been shown that these metals may enhance the antitumoral activity of TSCs, although they are considered to be major poison for living organisms. For example, it has been reported that several Pd(II) and Pt(II) complexes with methyl 2-pyridyl ketone thiosemicarbazone and *p*-isopropyl benzaldehyde thiosemicarbazone have potential antitumor activity [12].

The side effects of azomethin-H group in antimalarial drugs were showed with the increasing serum MDA level and serum activities of alanine transaminase (ALT), amilaz and superoxide dismutaze (SOD) activity in rats [15,16]. However, only a few reports are available *in vitro* and *in vivo* antioxidative, pro-oxidative properties [17-22]. In previous studies, we have investigated the effects on the some serum biochemical parameters (vitamins A,E,C and MDA) and morphological changes normal rat testes, liver and kidney of the ligand 4-(1-phenyl-methylcyclobutane-3-yl)-2-(2-hydroxybenzylidenehydrazino)thiazole (LH) and its Cd(II), Zn(II) and Cu(II) complexes [17,22]. As far as we know there are no studies related to effects on the serum amino acid levels of these compounds in literature.

In this study, we have investigated effects of the serum free amino acid levels of Pyridine-2-Carbaldehyde (LH) and its complexes formed with Cu(II) and Zn(II) ions (Fig 1.a,b). The synthesis, chemical and physical properties of these compounds have been reported [23].



(a)



(b)

Fig.1 (a) Structure of Pyridine-2-Carbaldehyde (LH) **(b)** suggested structure of the complexes of the Ligand.

The increasing interest to the mechanism in biological activity of ligands and metal complexes [18,19,24] and the metal-dependent oxidative damage to cells [25] have stimulated the present study, particullarly, the influence of the complexes including various metal ions on the biological activity have been investigated.

Materials and Methods

Animal treatment

The experiments were performed on male Wistar rats (190- 220 g). Rats were allowed free access to water and food. Room temperature was maintained at 24- 26 ^{o}C with a 12 h. light cycle. The animals were divided into seven groups, control and experimental groups. The animals were randomized in groups consisting of 5 animals in each group. After 2-3 days acclimatization, rats received subcutaneously injection of compounds (25 mg/kg body weight in a final volume of 500 µl corn oil + dimethylsulfoxide (DMSO)). The modified treatment method of compounds were used according to the previous studies [26-28]. The injections were repeated three days later throughout 15 days. For subcutaneously treatment, stock solutions of the compounds were made immediately before use. They were suspended in corn oil at the desired concentration following initial dissolution in 10 % DMSO. This concentration of DMSO by itself produced no observable toxic effects [29]. Vehicle treated rats served as controls. After the compounds were administered subcutaneously, the response manners have been observed on day 15th.

Survival testing

The toxicity of the 3 compounds were tested on male rats (group of 5 animals). The up and down method [30] were used to determine the median lethal dosage (LD_{50}) but LD_{50} values were not obtainable due to their poor solubility.

Sample collection

The experimental animals and controls were anaesthetized with ether and were sacrificied at the 15th day. Blood was withdrawn from the heart and collected in glass tubes without any anticoagulant and centrifuged at 4500 rpm for 10 minutes to use for determination of serum amino acid.

Instrumentation

The analyses were performed using a gas chromatograph Thermo Focus GC with FID, and equipped with an AI/A53000 autoinjector for liquid samples (Thermo, Rodano Milan, Italy) and data acquisition software ChromCard version 2.0 (Thermo). The column in use was ZebronTM ZB-AAA with dimensions 10m×0.25mm i.d. and a proprietary stationary phase

(Phenomenex, Torrance, CA, USA). The EZ:faastTM sample preparation kit was supplied by Phenomenex.

Analytical methods

Serum amino acid levels in this study were measured by Gas Chromatography (GC) with standart method [31].

Statistical analysis

Statistical analyses were performed with SPSS 10.0 for Windows software (SPSS Inc., Chicago, IL, USA). The limit of statistical significance was set at $P<0.05$. Results are expressed as mean_S.D. Comparison between mean values was made by One Way Anova variance analyses and means of two groups were compared by LSD test.

Results

Whilst the compounds administration, any mortality caused by ligands and complexes have not been observed. Serum amino acid levels are decreased by the compounds treatment (Table 1). We were unable to identify sufficient these results.

Table1. Serum amino acid concentrations from control and thiosemicarbazone derivatives and its metal complexes-treated rats

Groups (n=5)	Ala µM	Gly µM	Val µM	Leu µM	İle µM	Pro µM	Asn µM	Asp µM	Met µM
K	547,6±44,4	311,5±25,6	221,4±11,6	213,4±9,7	128,7±4,3	4941,2±215,4	729,3±89,1	53,4±9,8	73,5±9,8
LH	272,8±54,1^{c}	168,9±30,6^{c}	166,5±36,3^{b}	119,7±7,6^{c}	72,1±2,3^{c}	149,4±24,7^{c}	81,8±13,5^{c}	57,2±14,6	35,3±5,5^{c}
LH-Zn	299,3±16,2^{c}	178,9±22,2^{c}	171,9±25,1^{b}	121,0±10,8^{c}	73,8±1,9^{c}	159,4±16,0^{c}	81,6±14,5^{c}	46,2±10,1	49,8±5,0^{c}
LH-Cu	309,5±13,5^{c}	187,9±18,5^{c}	152,4±23,9^{b}	107,6±13,7^{c}	67,6±8,2^{c}	162,2±16,4^{c}	93,5±2,9^{c}	24,9±3,6^{c}	49,5±4,3^{c}

Groups (n=5)	Hyp µM	Glu µM	Phe µM	Gln µM	Ort µM	Lys µM	His µM	Try µM	Trp µM
K	21,3±2,5	257,7±57,2	81,2±3,9	457,2±72,3	157,1±52,3	432,3±29,8	63,8±6,0	80,0±4,5	125,9±12,6
LH	20,4±5,2	119,3±29,7^{c}	36,4±7,9^{c}	403,4±24,5	31,3±7,4^{c}	77,7±15,2^{c}	15,1±1,9^{c}	29,4±11,4^{c}	32,4±8,7^{c}
LH-Zn	21,2±6,1	101,7±11,2^{c}	39,7±4,3^{c}	296,1±64,6^{a}	35,0±6,9^{c}	159,7±29,8^{c}	17,8±3,1^{c}	33,6±6,3^{c}	68,6±13,7^{c}
LH-Cu	21,5±4,4	80,4±9,2^{c}	39,5±4,8^{c}	355,5±78,5	22,9±9,9^{c}	161,4±6,7^{c}	10,3±3,8^{c}	36,7±2,8^{c}	91,9±7,6^{c}

a: P< 0,05; b: P< 0,01; c: P< 0,001

Discussion

This work was carried out in order to verify the biological side effects of LH and metal complexes (LH-Zn and LH-Cu) and to clarify the contribution of effects on the serum amino

acid levels on the rats. For the first time determination of serum amino acid profiles of thioasemicarbazone-treated rats have been carried out in this study.

Byrnes et. al., reported that copper thiosemicarbazone complex caused oxidative stress by inducing free radical production [18]. Kaur and Ali [21], informed that 2-aril-3-[4-(substitue feril)-3-thiosemicarbazone] prevented the lipid peroxidation in rat liver. Likewise, thiosemicarbazone derivative caused a decrease in MDA level in diabetic rat liver cells [20].

The antitumor efficacy thiosemicarbazone derivatives against L1210 leukemia [32], and Friend erythroleukemia cells (FLC) [11] were investigated and were compared with respect to their activity on cell proliferation. They observed that ligand is active both in the inhibition of cell proliferation and in the induced differentiation but metal complexes have not possess significant antiproliferative activity [11]. Similarly, Pd(II) complexes of thiosemicarbazone were found to be active in the inhibition of DNA synthesis on Leukemia P388 cell cultures [14].

It has been recently reported that the ability of certain anticancer drugs to achieve a significant therapeutic index differentiating normal cells may be related to the fact that they induce apoptosis in tumor cells at drug concentrations significantly lower than those needed to kill normal cells [12].

As a result of these studies, it is suggested that thiosemicarbazone and Schiff base derivatives have wide biological effects. To our knowledge, the most of studies about thiosemicarbazone and its metal complexes made up to now are at biochemical level *in vitro* and on their antitumoral activity *in vivo* and *in vitro*. In addition, zinc and copper is very important for the metabolism. Zinc is an essential dietary factor that is present in all organs, tissues and body fluids and mediates a wide variety of physiological processes [33].

Copper is an essential element that contributes to important intracellular metabolic events [34], but is also a latent toxic element that leads to chemical element poison by excess accumulation [35] and is a potent hepatotoxin [36].

It has been reported that the structure and comformation of ligand has influence on the redox potential of central atom in coordination compounds [19]. The changes (coordination sphere) metal ions are connected with the change of diverse biological function of compound. The knowledge of these laws help us to synthesize more active complexes or to understand the

biological properties of natural biocoordinative compounds [19]. Fontana et.al reported that the serum amino acid levels were increased the cirrhosis induced by chronic administration of thioacetamide [37]. Serum amino acid concentrations from control and compounds-treated rats are shown in Table 1. Significant decreases in all amino acids were observed in rats.

In conclusion, the present results indicate that allcompounds caused decreasing the serum free amino acid levels was observed compared to control group. The explenation of behaviour of the compounds is more diffucult. We believe that the precise nature of the changes of biological activity of TSC and SB ligands and metal complexes need further investigation.

Acknowledgement

This study was financially supported by TUBITAK (project # 104T363).

References

1. Liberta AE, West DX. Antifungal and antitumour activity of heterocyclic thiosemicarbazones and their metal complexes. Biometals. 1992;5:121-6.
2. Wang LF, Zhu Y, Yang ZY, Wu JG, Wang Q. Manganase(II) complex of a hydrazone ligand derived from pyruvic acid and 4-pyridine carboxylic acid hydrazide-synthesis and crystal structure. Polyhedron. 1991;10:2477-81.
3. Padhy S, Kauffman GB. Transition metal complexes of semicarbazones and thiosemicarbazones. Coord. Chem. Rev. 1985;63:127.
4. Cory JG, Chiba P. Combination chemotheraphy directed at the components of nucleoside diphosphate reductase, in inhibitors of ribonucleoside diphosphate reductase activity. Eds.; Pergamon Press: Oxford, 1989; 245-64.
5. Cory JG, Cory AH, Rappa G, Lorico A, Liu MC, Lin TS, Sartorelli AC. Inhibitors of ribonucleotide reductase, comparative effects of amino and hydroxy- substituted pyridine-2-carbaxyaldehyde thiosemicarbazones. Biochem. Pharm. 1994;48(2):335-44.
6. Cory JG, Carter GL, Bacon PE, T'ang A, Lien EJ. Inhibition of ribonucleotide reductase and L1210 cell growth by N-hydroxy-N'-aminoguadine derivatives. Biochem. Pharm. 1985;34:2645-50.
7. Blanco JM, Caamano O, Fernandez F, Mera XG, Hergueta AR, Lopez C, Borgez JER, Balzarini J, Clercq E. Synthesis and antiviral and antineoplastic activities of some novel carbocyclic guanosine analogues with a cyclobutane ring. Chem. Pharm. Bull. 1999;47(9):1314-7.
8. Christophe SG, Caubere CK, Renard P, Pfeiffer B, Pierre A, Leonce S, Caubera P. Synthesis and antiproliferative activity of benzocyclobutacarbazol derivatives. A new class of potential antitumor agents. Bioorganic and Med. Chem. Let. 2000;10:2589-91

9. Dehmlow EV and Schmidt SS. Synthesis of stereoisomeric 3-substituted cyclobutanecarboxylic acid-derivatives. Liebigs Ann. Chem. 1990;5:411.
10. Agrawal KC, Mooney PD, Sartorelli AC. Potential antitumor agents 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone. J. Med. Chem. 1976;19:970-2.
11. Rodriguez-Argüelles MC, Ferrari MB, Fava GG, Pelizzi C, Pelosi G, Albertini R, Bonati A, Dall'Aglio PP, Lunghi P, Pinelli S. Acenaphthenequinone thiosemicarbazone and its transition metal complexes: synthesis, structure, and biological activity. J. Inorg. Biochem. 1997;66:7-17.
12. Perez JM, Matesanz AI, Martin – Ambite A, Novarro P, Alonso C, Savza P. Synthesis and characterization of complexes of p-isopropyl benzaldehyde and methyl-2-pyridyl ketone thiosemicarbazones with Zn(II) and Cd(II), metallic centers, cytotoxic activity and induction of apoptosis in pam-ros cells. J. Inorg. Biochem. 1999;75:255-61.
13. Demertzi DK, Domopoulou A, Demertzis MA, Valle G, Papageorgiou A. Palladium(II) complexes of 2-acetylpyridine N(4)-methyl, N(4)-ethyl and N(4)-phenyl- thiosemicarbazones. Crystal structure of chloro (2-acetylpyridine N(4)-methylthiosemicarbazonato) palladium(II). Synthesis, spectral studies, in vitro and in vivo antitumour activity. J. Inorg. Biochem. 1997;68:147-55.
14. Demertzi DK, Demertzis MA, Voragi V, Papageorgiou A, Mourelatus D, Mioglou E, Iakovidou Z, Kotsis A. Antineoplastic and cytogenetic effects of platinum(II) and palladium(II) complexes with pyridine-2-carboxaldehyde thiosemicarbazone. Chemoteraphy. 1998;44:421-6.
15. Novelli EL, Silva AM, Novellif JL, Curi PR. Reactive oxygen generation by azomethine H: a new antimalarial drug. Can. J. Physiol. Pharma. 1995;73(8):1189-94.
16. Novelli EL, Silva AM, Monteiro JP, Sacomani LB, Novellif JL. Free radical production by azomethine H: effects on pancreatic and hepatic tissues. Free Radic. Res. 1997;26(4):319-24.
17. Oner H, Karatepe M, Karatas F, Öner J, Yilmaz I, Cukurovalı A. Effects on the rat testes of thiosemicarbazone derivative schiff base (4-(1-phenyl-methylcyclobutane-3-yl)-2-(2-hydroxybenzylidenehydrazino) thiazole) and its cadmium (II) complex. Cell Biochem. and Funct. 2005;22:1-7.
18. Byrnes RW, Mohan M, Antholine WE, Xu RX, Petering DH. Oxidative stress induced by a copper thiosemicarbazones complex. Biochemistry. 1990;29:7046-53.
19. Durackova Z, Mendiola MA, Sevilla MT, Valent A. Thiohydrazone copper (II) complexes. The relationship between redox properties and superoxide dismutase mimetic activity. Bioelectrochemistry and Bioenergetics. 1999;48:109-16.
20. Hrnciarova M, Carsky J, Jakus V, Zlatos L, Kucharska J, Gvozdjakova A. Inhibition of lipid peroxidation in erythrocytes by aminoguanidine, resorcylidine aminoquanidine and their oxygen and sulfur analogs in diabetic rats. Bratisl. Lek. Listy. 1998;99(7):364-7.
21. Kaur S, Ali B. Membrane stabilization and inhibition of lipid peroxidation by anti - inflammatory indoles. Pharmacology. 1982;25(4):210-3.

22. Karatepe M, Karatas F, Antioxidant, pro-oxidant effect of the thiosemicarbazone derivative schiff base (4-(1-phenylmethylcyclobutane-3-yl)-2-(2 hydroxybenzylidenehydrazino) thiazole) and its metal complexes on rats. Cell Biochem. And Funct. 2006;24(6):547-54.
23. Balaban A, Sekerci M, Erk B. Synthesis, physico-chemical characterization, and stability constants of metal complexes of pyridine-2-carbaldehyde thiosemicarbazone. Synt. and React. Inorg and Metal-Org. Chem. 2003; 33(10):1775-86.
24. Stubbe J, Kozarich JW. Mechanism of bleomycin-induced DNA degredation. Chem. Rev. 1987;87(5):1107-36.
25. Aust SD, Morehouse LA, Thomas CE. Role of metals in oxygen radicals reactions. J. Free Rad. Biol. and Med. 1985;1:3-25.
26. Geran RI, Greenberg NH, Macdonald MM. et al. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (3rd ed.), Cancer Chemother. Reports (Part 3). 1972;3(2):1-103.
27. Anisimov VN, Khavinson V, Morozof VG. Carcinogenesis and aging. IV. Effect of low molecular weight factors of thymus, pineal gland and anterior hypothalamus on immunity, tumor incidence and life span of C3H/Sn mice. Mech. Aging Dev. 1982;19:245.
28. Kato T, Sato K, Kakinuma H, Matsuda Y. Enhanced suppression of tumor growth by combination of angiogenesis inhibitor O-(chloroacetyl-carbamoyl)fumagillol (TNP-470) and cytotoxic agents in mice. Cancer Research. 1994;54(19):5143-7.
29. Papageorgiou Z, Iakovidou DM, Mioglou E, Boutis L, Kotsis A, Demertzi DK, Domopoulou A, West DX, Demertzis MA. Antineoplastic and cytogenetic effects of complexes of Pd(II) with 4N-substituted derivatives of 2-acetyl-pyridine-thiosemicarbazone. Anticancer Research. 1997;17:247-52.
30. Kumamoto T, Toyooka K, Nishida M, Kuwahara H, Yashimura Y, Kawada J, Kubota S. Effect of 2,4-dihydro-3H-1,2,4-triazole-3-thiones and thiosemicarbazones on iodide uptake by the mouse thyroid; the relationship between their structure and antithyroid activity. Chem. Pharm. Bull. 1990;38(9):2595-6.
31. Mitja K, Irma VK, Mirko P. Determination of derivatized amino acids in human embryo culture media by gas chromatography. Journal of Chromatography B. 2007;858:292–5.
32. Liu CM, Lin TS, Sartorelli AC. Synthesis and antitumor activity of amino derivatives of pyridine-2-carboxaldehyde thiosemicarbazone. J. Med. Chem. 1992;35:3672-7.
33. Vallee BL, Falchuk KH. The biochemical basis of zinc physiology. Physiol Rev. 1993;73:79–118.
34. Hultberg B, Andersson A, Lsaksson A. Copper ions differ from other thiol reactive metal ions in their effects on the concentrations and redox status of thiols in HeLa cell cultures. Toxicology. 1997;117:89–97.
35. Shen SG, Lia H, Zhao YY, Zhang QY, Sun HW. The distribution patterns of trace elements in the blood and organs in a rabbit experimental model of copper pollution and study of haematology and biochemistry parameters. Env. Toxi. and Pharm. 2005;19:379–84.

36. Sokol RJ, Devereaux MW, Traber MG, Shikes RH. Copper toxicity and lipid peroxidation in isolated rat hepatocytes: effect of vitamin E. Pediatric Res. 1989;25(1):55-62.

37. Luis F, Eliane M, Maria IT, Maria IF, Antonio R, Fermin SM, Angel G. Serum amino acid changes in rats with thioacetamide-induced liver cirrhosis. Toxicology. 1996;106:197-206.