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Original Research

The determination of the effect of some 1,3,4 thiadiazole derivatives on biochemical content (Fatty Acids, Sterols, Lipophilic Vitamins) in rat liver

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Abstract: Thiadiazole derivatives and its metal compounds have antibacterial, antifungal, antitumoral, antiproliferative and antioxidant properties. In the study, the effects caused by thiadiazole ligand and its metal complexes upon the fatty acids and lipophilic vitamins in livers of rats were examined. The fatty acids in liver were specified by GC while the lipophilic vitamins were specified by HPLC. It was observed that the amounts of oleic acids (18:1, n-9) and monounsaturated fatty acids (MUFA) notably increased in the Mn complex group while the amounts of arachidonic acid (20:4, n-6) notably increased in the ligand group, compared to control group. The amounts of vitamin K_2, vitamin D_3 and α-tocopherol considerably increased in all groups compared to control group. It was noted that the amounts of α-tocopherol were elevated in both the Mn and Cr complex groups compared to control group. However, this elevation was matching with the amount in the same groups. Nevertheless, the amount of retinol was determined to be lower in the Mn complex group compared to other groups. Accordingly, it can be considered that thanks to the utilization of toxic metals such as manganese, cadmium and chrome, unsaturated fatty acids influenced the activities of the enzymes in liver tissue, which are in charge of fatty acid chain elongation.

Key words: Hydroxyurea Derivative 1, 3, 4-thiadiazole; Fatty acid; Lipophilic vitamin; Liver; Rat.

Introduction

Thiadiazole derivatives are utilized therapeutically for various diseases thanks to their antifungal (1), antiviral (2), antibacterial (3), anticonvulsant (4), antimicrobial and anti-inflammatory (5) properties. It was also reported that thiadiazole derivatives demonstrated antithyroid activities (6).

In a study conducted in-vivo, several thiadiazole derivatives (2,2-bis-1,3,4,-thiadiazole) were investigated and it was reported that these derivatives demonstrated antitumor and immunosuppressive activities against various types of leukemia cells such as L1210 leukemia, 6C3HED/OG lymphosarcoma, C1498 myeloid leukemia, Ehrlich carcinoma, sarcoma 180, B16 melanoma and against X5563 myeloma in BALB / 3T3 rats (7). Furthermore, the anti-proliferative activities of specifically N-substituted 2-amino-1, 3,4-thiadiazole derivatives were reported (8). In another in-vivo study (9), it was suggested that thiadiazole ligands inhibited the carbonic anhydrase enzyme (CA, EC 4.2.1.1). Additionally, they were reported to exhibit antioxidant activities by inhibiting lipid peroxidation (10) and protein oxidation (11). In another study, it was stated that the synthesized thiosemicarbazone derivatives exhibited antioxidant and pro-oxidant effects and the Schiff based Cd (II) metal derivative that contains thiosemicarbazone derivative caused damages in testicular tissue when injected at high doses (12). Furthermore, another study reported that Schiff base derivative's ligand that was synthesized from thiadiazole compounds, compared to the control group, did not cause oxidative stress and did not affect antioxidant parameters while Cu(L)2 caused a significant oxidative stress and Zn(L)2 acted as an antioxidant (13).

The aim of this study is to investigate the effects of several 1,3,4 thiadiazole derivatives on the fatty acids and lipophilic vitamins present in rat liver. In order to achieve this aim, ligand and thiadiazole-manganese, thiadiazole-cadmium and thiadiazole-chromium derivatives were used in the study.

Materials and Methods

Specimens

In this study, 35 male Wistar rats (14-16 weeks old, 200-220 g in weight) were experimented on. The specimens were permitted to acclimatize for two weeks following their arrival. The rats were kept in a temperature-controlled room (22-25 °C) with a 12:12 light-dark cycle; water and food were provided ad libitum. Experimental protocol approved by the Ethics Committee of Fırat University Animal Experiments was adopted. The study was conducted according to these rules. Specimens were divided into five groups as 1 control group (n=7) and 4 experimental groups (n = 7). Thia-

diazole compounds (TDAC) were administered to the experimental group. While only injections of 5 mL of 10%-below dimethylsulfoxide (DMSO) dissolved in corn oil were administered to control group (14), TDAC (25 mg kg^{-1}, 5 mL of 10% below DMSO dissolved in corn oil mixture) were injected to ligand and the other metal complex groups of rats, every other day. Injections lasted for 15 days with intervals lasting for three days during the experiment. All specimens were on a normal diet during the experiment. Blood samples were collected and stored at -20 °C until they were analyzed (15).

Chemicals

Hydroxyurea derivative 1, 3, 4 – thiadiazole compounds and their metal complexes, which were employed in the practices, were synthesized and characterized by the study of Cetin et al. (2006) (16).

Lipid extraction from the tissues

The process of extracting lipids from tissue and serum samples in the study were conducted by using the method of Hara and Radin (18) in which 3:2 (v/v) hexane-isopropanol mixture is used.

Preparation of fatty acid methyl esters from the tissues

In order to conduct a gas chromatographic analysis of the fatty acids present in lipids, they are required to be converted to their derivatives such as methyl esters that have non-polar, volatile and stable compositions. Although various methods are used for the conversion of fatty acids in lipids into their derivatives such as methyl esters, the method of acid catalysis esterification, as it was reported to have a practical application and high efficiency by Christie (140), was adopted in this study.

Gas chromatographic analysis of fatty acid methyl esters

Following the conversion of the fatty acids in the lipid extracts into methyl esters, the SHIMADZU GC 17 gas chromatography was analyzed. For conducting this analysis, the Machery-Nagel (Germany) capillary column with 25 m length, 0.25 μm inner diameter and PERMABOND 25-micron film thickness was used.

During the analysis, the column temperature was kept between 120-220 ºC while the injection temperature was kept at 240 ºC and detector temperature was kept at 280 ºC. The column's temperature program was set from 120 ºC up to 220 ºC. The increases in the temperatures were determined to be 5 ºC / min up to 200 ºC and 4 ºC / min up to220 ºC. The temperature was kept at 220 ºC for 8 minutes and the total time was determined to be 35 minutes. Nitrogen was used as the carrier gas. During the analysis, before the analysis of the fatty acid methyl esters of the samples, methyl esters of standard fatty acids were injected and the retention times for each fatty acid were determined. Following this process, the required programming was performed and the analyses of the fatty acid methyl ester mixtures' of the samples were conducted (39).

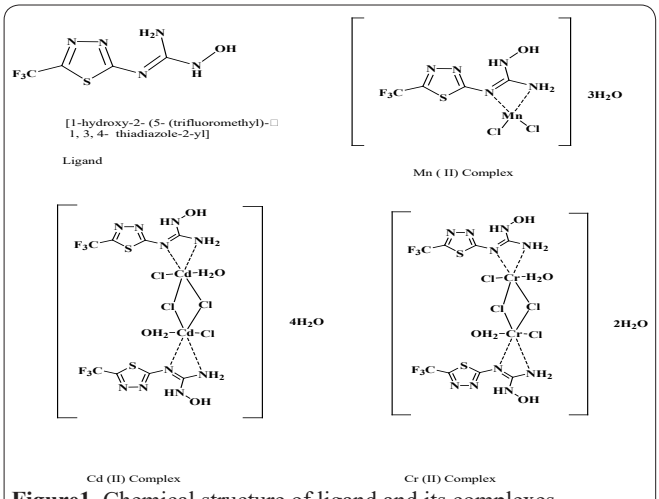

Figure1. Chemical structure of ligand and its complexes.

HPLC analysis of vitamins ADEK and sterol amount

5 ml supernatant was transferred into a 25 ml covered tube and a 5% KOH solution was added to it. Following the vortexing process, the tube was kept at 85 ºC for 15 minutes. Then, the tubes were ejected and cooled down to the room temperature. After that, 5 ml purified water was added on top and mixed. The unsaponified lipophilic molecules were extracted by using 2x5ml hexane. The hexane phase was evaporated by nitrogen flow. It was then solved by using 1 ml (50% + 50%, v/v) acetonitrile/methanol mixture and transferred to autosampler vials to conduct the analysis.

The analysis was conducted by using the Shimadzu branded HPLC device. In the device, LC-10 AD*VP* was used as the pump, CTO-10AS*VP* as the column oven, SIL-10AD*VP* as the autosampler and DGU-14A as the degasser unit, and *Class VP* software was employed (Shimadzu, Kyota Japan). Acetonitrile/methanol (60%+40%, v/v) mixture was used as the mobile phase. The mobile phase's flow speed was determined to be 1 ml. For the analysis, a UV detector was used. For the column, Supelcosil LC-18 (15x4.6 cm, 5 μm; Sigma, USA) column was used. The detection wavelength used for vitamin A was 326 nm while it was 202 nm for vitamin E and 265 nm for vitamin D and K (19).

Statistical analysis

The SPSS 15.0 (SPSS, Chicago, IL, USA) software program was used for statistical analysis. The comparison between the control group and experimental groups was made using ANOVA and LSD tests.

Results

Fatty acids

In liver tissues, palmitic acid (16:0) and docosahexaenoic acid (22:6,n-3) amount considerably elevated ($P < 0.05$, $P < 0.001$) in the ligand and Mn complex groups while the level of the fatty acids fell ($P < 0.01$) in the Cd and Cr complex groups compared to control group. Docosadienoic acid (22:2) ($P < 0.05$) and docosapentaenoic acid (22:5, n-3) ($P < 0.01$) amount marginally dropped in all groups compared to control group. Oleic acid (18:1, n-9) and monounsaturated fatty acids (MUFA) amounts were notably elevated in the Mn complex group while arachidonic acid (20:4, n-6) amount notably elevated in the ligand group compared to control

Table 1. Fatty acid composition of liver tissues.

Fatty Acids	Control	Ligand	Mn Complex	Cd Complex	Cr Complex
16:0	19,65±0,74	21,49±0,44[a]	20,92±0,25[a]	19,61±0,83[a]	19,10±0,36[a]
16:1	1,50±0,11	2,76± 0,17	2,39± 0,18	1,91± 0,18	2,16± 0,19
18:0	18,30±0,55	17,00± 0,88	16,71± 1,25	18,02± 1,00	16,68± 1,61
18:1	9,03±0,61	4,10± 0,17[b]	9,56± 1,95[b]	7,55± 1,47[b]	8,00± 2,11[b]
18:2	15,47±0,47[a]	15,23±0,80[a]	17,68±1,17[a]	17,99±1,06[a]	18,17±1,59[a]
20:3	0,85± 0,03[a]	0,95± 0,07[a]	0,84± 0,04[a]	0,76± 0,06[a]	0,90± 0,07[a]
20:4	26,02±1,16[b]	26,75±0,39[b]	22,56±1,58[b]	25,47±1,73[b]	24,25±2,05[b]
22:2	0,69± 0,02	0,48± 0,03[a]	0,54± 0,04[a]	0,61± 0,02[a]	0,50± 0,05[a]
22:5	1,28± 0,08	0,86± 0,02[b]	0,93± 0,04[b]	1,25± 0,15 [b]	1,18± 0,18[b]
22:6	2,96± 0,06	3,50± 0,16 [c]	2,99± 0,31[c]	2,43± 0,27[c]	2,90± 0,44[c]
24:0	0,40± 0,08	0,48± 0,05	0,46± 0,05	0,24± 0,02	0,43± 0,07
ΣSFA	38,35± 0,48	38,97± 0,43	38,09±0,49	37,87± 0,58	36,21± 0,67
ΣMUFA	10,53± 0,28	6.86±0,17[b]	11.95±0,50[b]	9.46±0,35[b]	10.16±0,29[b]
ΣPUFA	47,27± 0,79	47,27± 0,79	45,54± 0,81	48,51± 0,62	47,90± 0,73
Σ w - 3	4,24 ± 0,08	4,36±0,10	3,92±0,18	3,68±0,22	4,08±0,32
Σ w - 6	42,34±0,60	42,93±0,50	41,08±0,91	44,22±0,95	43,32±0,98

a: p<0.05, b: p<0.01, c: p<0.001.

group (P<0.01). Nevertheless, no significant difference was observed (P > 0.05) in the amount of palmitoleic acid (16:1, n-7), stearic acid (18:0), lignoceric acid (24:0), total saturated fatty acids (SFA) and, total omega-3 and omega-6 fatty acids among groups, polyunsaturated fatty acids (PUFA). Whereas the amount of linoleic acid (18:2, n-6) was determined to be lower (P<0.05) in the ligand group compared to other groups, the amount of eicosatrienoic acid (20:3, n-6) was observed to be lower (P < 0.05) in the Mn complex group compared to the other groups (Table 1).

Lipophilic vitamins

There was no notable difference (P>0.05) considering the amount of cholesterol and K_1 vitamin among the groups. The amount of vitamin K_2, vitamin D_3 and α-tocopherol were notably elevated (P<0.05) in the all groups compared to control group. It was determined that the amount of α-tocopherol increased both in the Mn and Cr complex groups compared to control group. However, the increase was found to be matching for the amount in the same groups (P<0.05). Vitamin D_2 amount was observed to be lower in the Cd and Mn complex groups compared to other groups (P<0.05). However, the amount of retinol was observed to be lower (P<0.05) in the Mn complex group compared to other groups. In the ergosterol amount, it was noted that its amount was decreased in the Cd and Mn complex groups compared to other groups (P<0.05) (Table 2).

Discussion

The biological properties of thiadiazoles are usually associated with ion coordination. The metal complexes often exhibit similar bioactivities, which are not exhibited by the free ligands, and some of the side effects along with drug resistances may decline upon complexation (21).

In this study, vitamins K1, K2, D2, D3, Retinol and α-tocopherol in liver were investigated in terms of treatments with compounds and complexes of thiadiazole. It was determined that the levels of vitamins K1, K2, D2, D3, Retinol and α-tocopherol were altered by conducting treatments with thiadiazole compounds and complexes. It was also noted that all of the complexes increased the levels of vitamin A, K1, K2, D3 and α-tocopherol. Furthermore, Mn and Cd complexes decreased the levels of vitamin D2 while ligand and Cr complex increased the levels of vitamin D2 in liver tissue. Although ligand, Cd and Cr complexes increased the levels of Retinol, Mn complex decreased the Retinol level. Additionally, it was determined that Ligand and Cr complexes increased Ergosterol levels while Mn and Cd complexes decreased Ergosterol. All of the complexes, except for the Cr complex, decreased the cholesterol levels. Ac-

Table 2. Lypophilic vitamins composition in liver tissues.

Vitamins	Control	Ligand	Mn Complex	Cd Complex	Cr Complex
Cholesterol	1028±107,7	840,1±77,4	827,1±95,04	771,2±27,5	1096,9±62,4
K1	2,16±0,28	4,70±0,68	3,11±0,80	3,28±0,32	3,98±0,50
K2	3,51±0,37	4,90±1,22[b]	6,06±1,12[b]	7,73±2,9[b]	5,43±0,67[b]
D2	0,22±0,15	1,91±1,42[c]	0,17±0,16[c]	0,15±0,00[c]	0,93±0,78[c]
D3	0,26±0,12	0,71±0,40[a]	0,32±0,01[a]	3,06±3,18[a]	0,47±0,08[a]
α-tocopherol	9,37±0,42	18,63±2,43[a]	11,47±2,7[a]	10,62±0,7[a]	11,73±3,2[a]
Retinol	332,8±11,3	400,58±9,5[b]	257,9±127,3[b]	354,9±25[b]	400,8±43[b]
Ergosterol	3,56±0,40	13,73±2,27[a]	2,65±0,32[a]	2,71±0,08[a]	4,63±0,57[a]

a: p<0.05, b: p<0.01, c: p<0.001.

cording to these results, it was determined in the study that the studied compounds and especially their ligands caused improvements in the antioxidant capacities in tissues by increasing the antioxidant vitamin levels.

Studies with different results were found in the literature. This may be due to the different ligand structure. In a study, Turan et. al. reported that 1,3,4-thiadiazole-based Schiff base and its metal complexes did not change serum vitamins A, E, and C levels. However, they reported that vitamin levels were lower in treatment groups, compared to the control group in the liver tissues (22). In another study by Karatepe and Karatas (13) found that serum vitamins A, E concentrations were statistically changed but serum level of vitamin C was not changed. Due to the different structure of the ligand results may be different.

According to the results of our study, it was observed that thiadiazole-manganese, thiadiazole-cadmium and thiadiazole-chrome complexes from complex groups increased the amount of linoleic acid in the liver compared to control group. In a previous study, it was reported that the Δ-9 activity of desaturase is prevented with the cadmium application (23). In the study conducted by Dayangaç et al. (2006) the effects of cadmium on fatty acid compositions were investigated in some tissues (testis, liver, heart and kidney tissues) of rats and it was reported that C16:0 and C18:0 amount of cadmium group decreased while C18:1 (n-9), 18:2 (n-6), C20:4 (n-6), 22:6 (n-3) amount increased compared to control group in liver. C18:1 (n-9) and C18:0 amount of cadmium group increased compared to control group in kidney ($P<0.05$). 18:1 values of the cadmium group in testis tissues decreased while C20:4 (n-6) acid value increased (24).

18: 2 (n-6), 18: 3 and C 20: 4 (n-6) unsaturated fatty acids are known as essential fatty acids and they are of vital importance for the metabolisms of organisms. It is also known that long-chain unsaturated fatty acids are sensitive to the disruptive effects of peroxide and molecular oxygen. In a study, it was reported that the increase in the lipid peroxidation in the tissues were caused by the catalytic peroxidation of various metal ions and linoleic acid (25).

In another study, l-(lmesityl- l-methylcylobutane-3-yl)-2-succinoimido ethanone thiosemicarbazone (MSTSC) and l-(l-phenyl-lmethylcylobutane-3-yl)-2-succinoimido ethanone thiosemicarbazone (FSTSC) derivatives were investigated in terms of their effects on rabbit liver and it was reported that C16: 0, C18: 0 fatty acid levels were decreased in MSTSC and FSTSC groups ($p <0,01$) while C18: 2 and C18: 1 fatty acid levels were increased compared to the control group. Additionally, in thiadiazole ligand group, arachidonic acid was increased and linoleic acid was decreased compared to control group (26). Arachidonic acid is synthesized with 4^6 desaturase and 4^5 desaturases enzymes of linoleic fatty acid (27). The decrease observed in the level of linoleic compared to control group and the increase observed in the level of arachidonic acid compared to control group may be an indicator of which the way of 4^5 desaturase, one of these specified enzymes, is very active. As it is known, long-chain unsaturated fatty acids have desaturase enzymes, which are responsible for the chain elongation reaction.

In conclusion, it is believed that by applying toxic metals such as manganese, cadmium and chrome, unsaturated fatty acids affected the activities of enzymes in the liver tissue, which are in charge of the fatty acid chain elongation.

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