M W Journal of Science

ISSN: 3023-770X

https://doi.org/10.5281/zenodo.15252058 MW Journal of Science, 2025, 2, 1, 1-8

An Investigatıon of the Effects of 2-Aminoimidazoline Derivative on the Levels of Antioxidant Vitamıns (A, E, and C) and MDA of Rats

Eyüp Andaç KARATAŞ[1], Refik SERVİ[2], Erkan YÜKSEK[3], Haki KARA[4], Fikret KARATAŞ[3]*

[1] Fethi Sekin City Hospital Urology Department, Elazığ, TÜRKİYE
[2] Fırat University, Faculty of Medicine, Department of Anatomy, Elazığ, TÜRKİYE
[3] Firat University, Faculty of Science, Department of Chemistry, 23119 Elazig, TÜRKİYE
[4] Cumhuriyet University Faculty of Veterinary Medicine, Department of Pharmacology, Sivas, TÜRKİYE
*Corresponding author: fkaratas@firat.edu.tr

Abstract

This study was carried out to determine the amounts of malondialdehyde (MDA) and vitamins A, E, and C, which are indicators of lipid peroxides, in the blood serum, liver, and kidney tissues of rats given N-(5-phenyl[1,3,4]-thidiazol-2-yl)-N-(4,5-dihydro-1H-imidazol-2yl) amine using high performance liquid chromatography (HPLC).

2-Aminoimidazoline derivative was injected into the 9 rats of 25 mg kg-1 (dissolved in 250 µL of %75 ethanol). For the control group, only 250 µL of %75 ethanol was injected into the other 7 rats for 16 days (every other day). Afterward, all rats failed using ether and blood serum, and liver and kidney tissues were taken.

It was found that the level of C vitamin in rats under the skin of which we injected thiadiazolimidazoline was lower than that in the control group ($p<0.005$). The same results were observed for A and E vitamins in serum and for A, E, and C in liver and kidney tissues ($p<0.05$).

It was observed that MDA levels of the liver and serum of rats in which the 2-aminoimidazoline derivative was injected were higher than in the control groups (p□0.005). Moreover, this level was higher in the rats under the skin, for which we injected 2-amino imidazoline derivative, than in the control groups for kidney tissues ($p<0.05$). These observations show that the production of free radicals was increased by the 2-amino imidazoline derivative, producing stress in rats. Because of the same reason, the antioxidant level was decreased.

In conclusion, using antioxidant vitamins with drugs containing any 2-amino imidazoline derivative is beneficial.

Keywords: 2-Aminoimidazoline derivative, Vitamin A, Vitamin E, Vitamin C and MDA, Rat

Introduction

Benzimidazole and imidazole ring systems have practical pharmacological effects in medicinal chemistry. 2-Substituted-1H-benzomidazoles and 2-substituted-1H-imidazoles, as well as 2-arylamino imidazoline derivatives, have also found broad application areas in medicinal chemistry in recent years [1], [2]. Aminoimidazoline derivatives are frequently added as additives to drugs used in hypertension [3]. Isoniazid, one of the chemicals containing the imidazole group, is a structure used in diagnosing tuberculosis. Isoniazid creates resistance in the body against tuberculosis bacteria and prevents the bacteria's activity [4]. Derivatives of 2-arylamino-2-imidazoles have a blood pressure-lowering effect on

the cardiovascular system and are used as analgesics and sedatives in treatments and surgeries. It has been determined that they are used as antidiuretic substances and also have anti-diarrheal properties. They have intraocular eye pressure-lowering effects. α2-Adrenoreceptor against. It has a neurosedative effect, and indanozoline is a beneficial vasoconstrictive substance [5].

Benzimidazole derivatives bearing phenyl substitution at position 2 of the phenyl ring are reported to have analgesic [6], anti-inflammatory [7], antimicrobial [8], spasmolytic [9], antiviral [10], anthelmintic [11] activities. 1-(β-dimethylaminoethyl)benzimidazole derivatives have been proven to have morphine-like analgesic activity [12]. Serotonin, an imidazole derivative, is one of the chemicals involved in nerve transfer. This structure plays an active role in the regulation of the cardiovascular system. It is also effective in regulating memory, sleep, and nutrition. Studies on platelet coagulation inhibitors and compounds with fused or single imidazole rings and the relationship between their activities and the importance of lipophilic particles are discussed [13]. The antiplatelet and vasodilator effects of 5-methyl-4-(3-pyridyl)-2-(substituted benzimidazole-5-yl) imidazole derivatives have been studied. They have been observed to inhibit enzymes in the platelet coagulation cascade, such as thromboxane A2 synthetase, phosphodiesterase (PDE), and cyclooxygenase [14]. N-mono N-N disubstituted dithiocarbamate derivatives have antibacterial and antifungal activity against bacteria and fungi. Methyl N-arylthiocarbamates and dimethyl N-aryl dithiocarbonimide compounds have interesting chemistry and enable the synthesis of 2-arylamino-2-imidazoline derivatives clonidine and maxonidine, which are well known in the literature [15]. Bis (2-aminoimidazoline) chemical is used in treating Human African Trypanosomiasis, HAT, also known as sleeping sickness. In addition, it has been proven by previous studies that imidazole structures are used as active ingredients in drugs used in the treatment of this disease. N, N'-bis(4-aminophenyl) piperazine is one of the imidazole derivatives used for this purpose [16].

Aminoimidazolines are found in many drugs. Therefore, it was aimed to determine the effects of the original synthesized and characterized N-(5-phenyl [1,3,4]-thiadiazol-2-yl)-N-(4,5-dihydro-1H-imidazol-2yl) amin on the levels of antioxidant vitamins (A, E and C) and malondialdehyde (MDA), a product of lipid peroxidation, in rats and to investigate the relationships between these parameters.

2. Materıal and Methods

2.1. Animal Material

In our research, by the ethical committee decision numbered 65202830-050.04.04.-12 taken at Sivas Cumhuriyet University, 12-14-week-old adult Wistar male rats weighing 250 grams were used. A 12-hour light and 12-hour dark environment was prepared for the animals we used in our experiments. Again, the ambient temperature was kept constant at room conditions (24-26 ˚C). The applications were made at the same time. The rats used in the applications were constantly checked from birth, and rats that could be suspected of having a disease were not included in the groups. No rat deaths were observed during the experiments.

2.2. Creating Groups

Sixteen rats, 7 in the control group and 9 in the treatment group, were used, and the 2-amino imidazoline derivative was dissolved in 75% ethyl alcohol. A 25 mg kg-1 dose of 250 L was injected subcutaneously into the rats every other day. Similarly, 250 µL of 75% ethanol was injected subcutaneously into the control group every other day. In this study, the original 2-aminoimidazoline derivative [1], whose formula is shown, was used, which was synthesized and characterized.

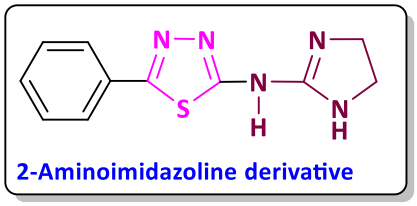

Figure 1. The structure of N-(5-Phenyl [1,3,4]-thiadiazol-2-yl)-N-(4,5-dihydro-1H-imidazol-2yl) amine.

2.3. Collecting Blood and Tissue Samples

After the applications, the animals were anesthetized with ether, and their rib cages were opened. A sufficient amount of blood (3-5 mL) was taken from their hearts. Blood samples in polyethylene tubes were centrifuged, and their serums were separated. The separated serums were analyzed within three days at the latest. Serum was stored in polyethylene tubes, and liver and kidney samples were wrapped in aluminum foil and stored at -20 ˚C until analyzed.

2.4. Preparation of Tissue Samples (Liver and Kidney)

After homogenizing the liver and kidney samples in the homogenizer, 0.2 g were weighed, and the same procedures were applied to the serum and tissue samples.

2.5. Methods

2.5.1. Determination of Vitamin A and E

After the serum, liver, and kidney samples taken from the deep freezer were thawed at room temperature, 0.3 mL of the serum sample and the liver and kidney tissues were thoroughly homogenized in a homogenizer, and 0.2 grams of each were weighed and placed in each plastic tube. 2 mL of ethanol was added to each tube to precipitate the proteins. 0.3 mL of n-hexane was added to the filtrate, separated by filtration, and mixed in a vortex. Then, the upper hexane phase was separated by centrifugation at 4000 rpm for 3 minutes. The hexane extraction process was repeated twice for each sample. Thus, all vitamins A and E in the serum were taken into the hexane phase. The separated hexane phase was removed under nitrogen gas until dryness. Then, the samples were dissolved in 0.15 mL methanol again and made ready for analysis. 20 µL of this methanol solution was taken and injected into HPLC. In HPLC, a mixture of methanol: acetonitrile: chloroform (47: 42: 11) was used as the mobile phase in Techsphere ODS-2 (5 mm, 250 x 4.6 ID) [17].

2.5.2. Determination of Serum Vitamin C and MDA Levels

From the samples warmed to room temperature, 0.3 mL of serum and 0.25 grams of homogenized liver and kidney samples were weighed and placed in plastic tubes. Serum samples were vortexed for 2 min with 0.3 mL of 0.5 M HClO4, liver and kidney samples were each vortexed for 0.5 mL of 0.5 M HClO4, and proteins were precipitated. Then, all samples were kept in an ultrasonic water bath at 50 ºC for 5 min to ensure thorough disintegration. Then, the total volume of serum samples was made up to 2 mL with distilled water, and the volume of liver and kidney samples was made up to 5 mL with distilled water and centrifuged at 4500 rpm for 10 min. Separation of precipitate and filtrate was ensured from the centrifuged solution. The Supelcosil LC-18-DB HPLC reversed-phase column (3 µm particle size and 250×3.9 ID) was utilized to detect vitamin C and MDA levels. Mobile phase 30 mM KH2PO4 buffer, pH=4 with H3PO4 and methanol (65–35% v/v) at 1.5 mL min−1 flow rate [17], [18].

2.6. Statistical analysis

Results are given as mean ± deviation. Findings were subjected to One-Way ANOVA using SPSS 26.0 for MS Windows. Differences between group means were analyzed for significance using the Tukey HSD test, and statistical significance was expressed as $p<0.05$.

3. Results and Discussion

It is not well known today that oxidative stress, characterized by the unusual and intense production of species resulting from oxygen reduction in many cell types during various pathological conditions, occurs. A general consequence of this oxidative stress is the peroxidation of cell lipids, resulting in a greater or lesser disintegration of the cell organization [19]. Lipid peroxidation structures have free radicals that cause degeneration of cell membranes. These free radicals affect important cell structures such as fat, protein, carbohydrate, and nucleic acid in the cell structure [20]. It is reported that many diseases are caused by free radicals [21]. Vitamin A, one of the antioxidant substances, performs its antioxidant activity by preventing the formation of free radicals and collecting radicals in the environment [22]. Vitamin E shows antioxidant activity by destroying free radical species in the early stages of lipid peroxidation or by preventing their formation and by stabilizing free radicals and breaking the peroxidation chain [23]. It is reported that Vitamin C can show its antioxidant effect by clearing singlet oxygen, superoxide, hydroxyl, hydroperoxyl, lipid peroxyl, and alkoxyl radicals from the environment [24]. Lipid peroxidase structures deteriorate quickly in the cell and form reactive carbon compounds. MDA, one of the essential carbon structures formed, is an indicator in the lipid peroxidation system [25].

As shown in Figure 2, the serum antioxidant vitamin A and E levels of rats injected with 2-aminoimidazoline derivative were lower ($p<0.05$) compared to the control group. In contrast, vitamin C levels were lower ($p<0.005$) than the control group. It was observed that the MDA levels of the 2-aminoimidazoline derivative group increased compared to the control group ($p<0.005$).

Karatas et al. [26] reported that a subcutaneous injection of (5-bromobenzofuran-2-yl) (3-methyl-3-mesitylcyclobutyl) ketonethiosemicarbazone at a dose of 25 mg kg–1 caused a decrease in the amounts of vitamins A, E and C and an increase in the amount of MDA in the serum of rats compared to the control group.

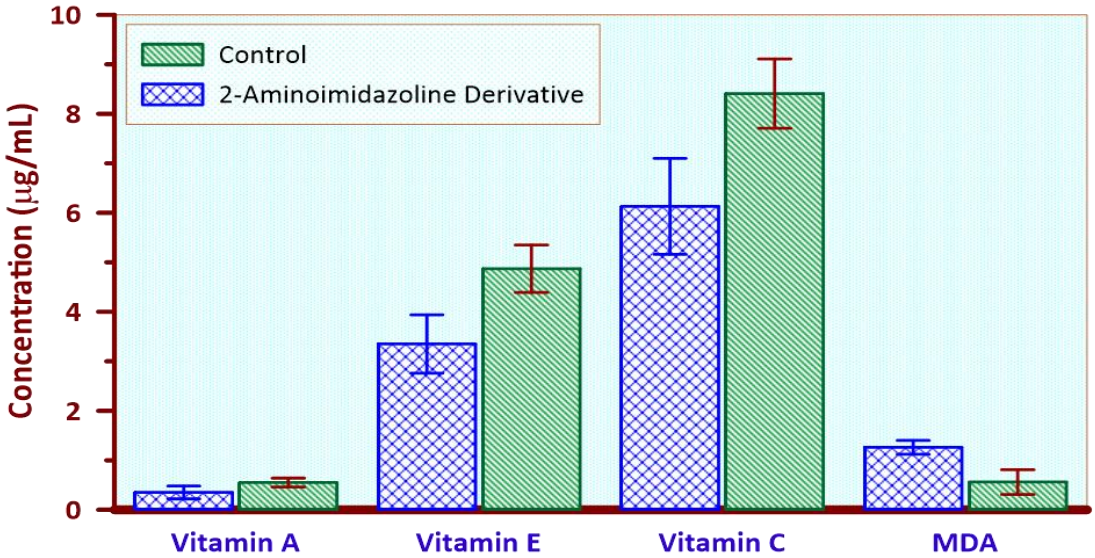

Figure 2. Graph of serum antioxidant vitamins (A, E, and C) and MDA levels of the control group and 2-aminoimidazoline injected rats

As seen in Figure 3, the amounts of liver antioxidant vitamins (A, E, and C) in rats injected with 2-amioimidazoline derivative were determined to be lower ($p<0.05$) than in the control groups. It was observed that the amounts of MDA increased ($p<0.005$) compared to the control group.

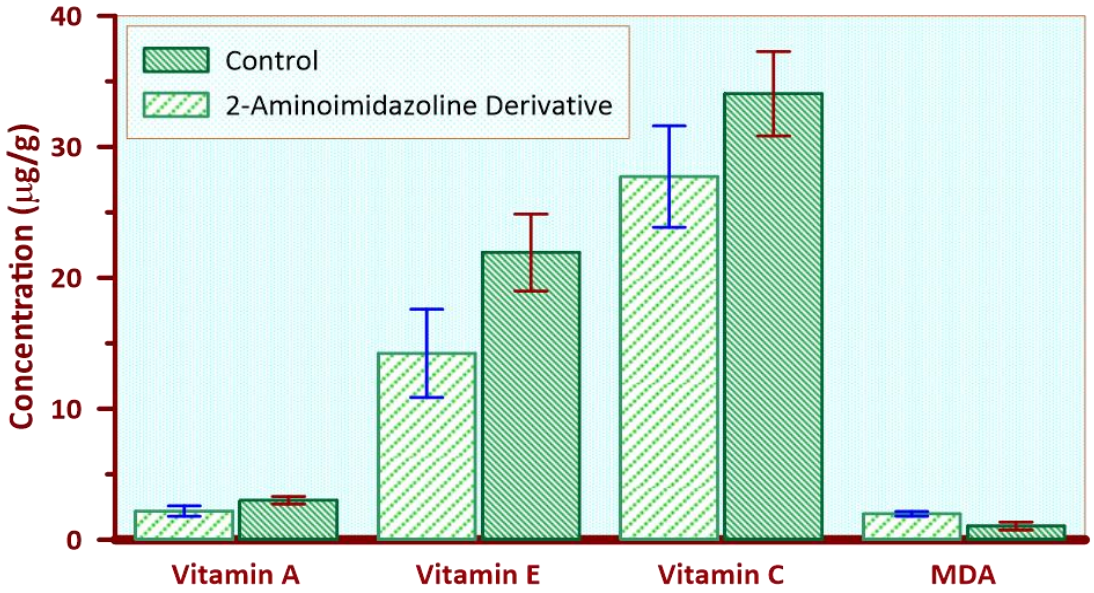

Figure 3. Graph of antioxidant vitamins (A, E, and C) and MDA levels in rats injected with control group liver tissue and 2-aminoimidazoline derivative.

Karatas et al. [17] reported that the amounts of vitamins A, E, and C in the serum of rats injected with 2-Furan-2-yl-1H-Benzimidazole by subcutaneous injection every second day decreased compared to the control group, while the amount of MDA increased.

As seen in Figure 4, the amounts of kidney antioxidant vitamins (A, E, and C) in rats injected with 2-amioimidazoline derivative were found to be lower ($p<0.05$) than in the control groups. The MDA amounts were increased ($p<0.05$) compared to the control group.

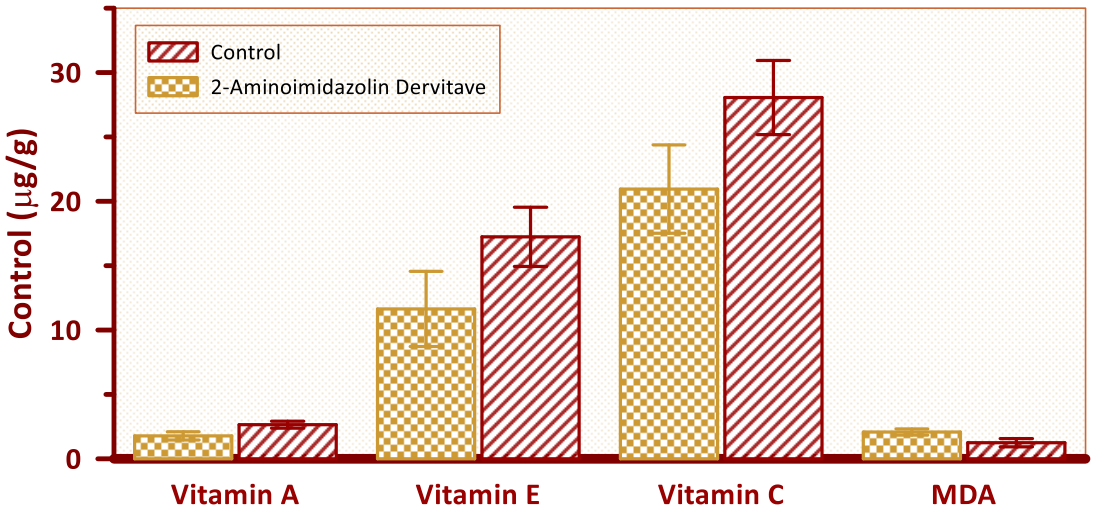

Figure 4. Graph of antioxidant vitamins (A, E, and C) and MDA levels in kidney tissue of the control group and 2-aminoimidazoline injected rats.

In our findings, the serum, liver, and kidney MDA levels of the rats injected with 2-aminobenzimidazoline were higher than in the control group. This is an indication of oxidative stress. Vitamin E stops the lipid

peroxidation reaction by holding the free radicals in the environment. In this system, the α-tocopherol structure is transformed into the α-tocopherol radical. Vitamin C forms α-tocopherol again by acting from α-tocopherol [27].

Çöteli et al. [28] subcutaneously injected 250 µL of 10% DMSO solution dissolved in corn oil into the control group of rats for 30 days. In the experimental group, 20 mg kg-1 250 µL of 1H,1'H-3,3'-biindazole dissolved in 10% DMSO was administered simultaneously daily. At the end of the study, they reported a decrease in the amount of vitamins A, E, and C and an increase in the amount of MDA in the serum, liver, and kidney tissues of the rats compared to the control group.

Vitamin E, one of the essential antioxidants, is carried by selenoproteins and cleans the free radicals in the environment. A decrease was determined in the serum liver and kidney vitamin A, E, and C levels of the rats injected with 2-aminobenzimidazoline compared to the control group. Vitamin C prevents the formation of nitrosamine structures from nitrite and nitrate [29].

In the study, the undesirable changes observed in antioxidant vitamins in the serum and liver, including the kidneys, may be due to possible free radical attacks. This shows that free radicals are formed in excess, exceeding the protective effects of antioxidant defense mechanisms. The findings show that 2-aminobenzimidazoline increases the production of free radicals by creating oxidative stress and that due to this increase in radicals, lipids undergo peroxidation and increase the amount of MDA, which is one of the end products. As it is known, antioxidant vitamins neutralize free radicals. The increase in MDA in rats' liver, kidney, and blood serum can be explained by the consumption of antioxidant vitamins in neutralizing free radicals.

The changes observed in malondialdehyde and vitamins indicate that oxidative stress-related destruction may have occurred in the cells. It is reported in the literature that there will be an increase in peroxidation products and a decrease in glutathione amounts due to lipid peroxidation resulting from oxidative damage [30]. The findings obtained in our study indicate that 2-aminobenzimidazoline also causes oxidative stress because an increase in MDA levels was observed in the serum, liver, and kidney. This situation is consistent with the information in the literature.

4. Conclusion and Suggestions

When 2-Aminoimidazole derivative, which is found in the structure of many drugs, was injected into rats, it was observed that the amount of antioxidant vitamins (A, E, and C) decreased in the rats' blood serum, liver, and kidney tissues. In contrast, the amount of MDA, a lipid peroxidation product, increased. It is known that 2-aminobenzimidazoline, which was used in this study, is also found in the structure of some drugs. Therefore, taking antioxidant vitamins and using drugs containing 2-aminobenzimidazoline or its derivatives may be beneficial.

Contributions

All the authors have contributed equally.

Conflict of Interest Statement

The authors declare that they have no known conflict of interest.

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