KLİNİK ÇALIŞMA / CLINICAL TRIAL
Ege Klin Tıp Derg 2023;61 (1): 64-68

Antioxidant Efficacy Of Astaxanthin On Amiodarone Induced Toxicity in Rat

Amiodaron'un Neden Olduğu Sıçan Doku Toksisitesinde Astaksantin'in Antioksidan Etkinliği

Özlem KARA*0000-0002-2084-8290
Asuman KİLİTCİ0000-0002-5489-2222**

* Kirsehir Ahi Evran University School of Medicine, Department of Histology and Embryology, Kirsehir, Turkey,
** Duzce University School of Medicine, Department of Pathology, Duzce, Turkey

Corresponding author: Ozlem KARA
Kirsehir Ahi Evran University School of Medicine, Department of Histology and Embryology, Kirsehir, Turkey
E mail: ozlemozturk34@hotmail.com

Geliş Tarihi: 29/12/2022
Kabul Tarihi: 16/02/2023

Abstract

Aim: We aimed to evaluate the effect of astaxanthin on amiodarone induced kidney tissue damage.

Methods: 3 groups were formed using 30 Wistar albino rats. In group 1 (control group) (n=10), neither any drugs were given nor anything was performed. In group 2 (amiodarone group) (n=10), 100 mg/kg amiodarone was given for 7 days. In group 3 (amiodarone+astaxanthin group) (n=10), 100 mg/kg amiodarone and 25 mg/kg astaxanthin were given for 7 days. Right kidneys were surgically extirpated in all groups. Blood malondialdehyde (MDA) levels and activities of catalase (CAT) and superoxide dismutase (SOD) were measured. Also, toxicity markers such as vascular congestion, hemorrhage, tubule degeneration and glomerular damage were assessed by examining the slides prepared from kidney tissue with microscopy.

Results: The MDA levels were significantly higher and the activities of SOD, and CAT were lower in group 2 than group 3 ($p<0.05$). Tissue damage was significantly higher in group 2 than group 3 ($p<0.05$).

Conclusion: According to our short term findings, astaxanthin reversed the toxicity of amiodarone on kidney tissue.

Keywords: Amiodarone, astaxanthin, rat, kidney, toxicity

Öz

Amaç: Astaksantin'in amiodaronun neden olduğu böbrek dokusu hasarı üzerindeki etkisini değerlendirmeyi amaçladık.

Yöntemler: 30 adet Wistar albino rat kullanılarak 3 grup oluşturuldu. Grup 1'de (kontrol grubu) (n=10) herhangi bir ilaç verilmedi ve herhangi bir işlem yapılmadı. Grup 2'ye (amiodaron grubu) (n=10) 100 mg/kg amiodaron 7 gün verildi. Grup 3'e (amiodaron+astaksantin grubu) (n=10) 100 mg/kg amiodaron ve 25 mg/kg astaksantin 7 gün verildi. Tüm gruplarda sağ böbrekler cerrahi olarak çıkarıldı. Kan malondialdehit (MDA) seviyeleri ve katalaz (CAT) ve süperoksit dismutaz (SOD) aktiviteleri ölçüldü. Ayrıca böbrek dokusundan hazırlanan lamlar mikroskopi ile incelenerek damar tıkanıklığı, kanama, tübül dejenerasyonu ve glomerüler hasar gibi toksisite belirteçleri değerlendirildi.

Bulgular: Grup 2'de MDA düzeyleri grup 3'e göre anlamlı olarak daha yüksek, SOD ve CAT aktiviteleri daha düşüktü ($p<0.05$). Doku hasarı grup 2'de grup 3'e göre anlamlı olarak yüksekti ($p<0,05$).

Sonuç: Kısa vadeli bulgularımıza göre astaksantin, amiodaronun böbrek dokusu üzerindeki toksisitesini tersine çevirmiştir.

Anahtar Kelimeler: Amiodaron, astaksantin, sıçan, böbrek, toksisite

Introduction

Amiodarone, an iodine-rich benzofuranic derivative, is currently used as an antiarrhythmic agent in the treatment of arrhythmias such as ventricular arrhythmias, paroxysmal supraventricular tachycardia, and atrial fibrillation (1). Amiodarone causes a number of histological changes, including intertubular leukocyte infiltration, degeneration of the renal tubules, and glomerular atrophy (2). The inhibition of the liposomal phospholipase enzyme by amiodarone results in an increase in phospholipid and free radical levels and cell death occurs (3). The high iodine content of amiodarone limits its use because it causes adverse effects on the thyroid and other tissues (4).

Astaxanthin is an important carotenoid pigment found in microalgae, mushrooms, seafood, flamingos and quails used in the food, cosmetics and feed industries (5). In addition to the very strong antioxidant properties of astaxanthin; it is thought to have many properties that are protective against ultraviolet (UV) light photo-oxidation, anti-inflammatory, anticancer, antidiabetic, anti-ulcer due to Helicobacter pylori, immunomodulatory, aging and age-related diseases, or healing on liver, heart, eye, joint and prostate (6, 7). Astaxanthin exhibits strong antioxidant effect due to its oxygen content. In a study in which a diabetic retinopathy model was created, astaxanthin increased the level of oxygenase 1 enzyme and restored homeostasis in the cell (8). It is known that astaxanthin has been used successfully in the treatment of many pathologies such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Amyotrophic Lateral Sclerosis, especially neurological disorders (9, 10). Therefore, we aimed to reverse the kidney damage in rats given amiodarone with astaxanthin.

Material and Methods

In this study, it was investigated whether astaxanthin was effective in kidney damage due to amiodarone in Wistar Albino rats. The amiodarone and astaxanthin used in the study were obtained from a local pharmacy. A total of 24 Wistar albino rats were included in the study. Animals were obtained from Erciyes University Animal Experiments Department. The study was carried out in Erciyes University Faculty of Medicine, Department of Histology and Embryology. Ethical approval of the study was obtained from Erciyes University Animal Experiments Local Ethics Committee. Rats were fed ad libitum feeding method with free access to water and food, and were exposed to a temperature of 20-22 C and a 12-hour light/dark period.

A total of 3 groups were created. The groups and given drugs are shown in **Table 1.**

Table 1. Experimental groups and given drugs

Number of the groups	Groups	Number of the patients	Amount of the substance
1	Control group	10	None
2	Amiodarone group	10	amiodarone (100 mg/kg/day) 7 days
3	Amiodarone+astaxanthin group	10	amiodarone (100 mg/kg/gün 7 days+ astaxanthin 25 mg/kg/day (350 µl dissolved in olive oil, oral) 7 day

Ketamine hydrochloride (50 mg/kg, Ketalar, Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride (5 mg/kg, Rompun, Bayer, Leverkusen, Germany) were administered intraperitoneally for anesthesia. Blood was collected from rats by cardiac puncture. Then, the right kidney tissues were surgically removed and the animals were sacrificed by cervical dislocation.

Tissues were fixed in formaldehyde solution and then embedded in paraffin. Sections of 5 µm in diameter were taken. Sections were stained with hematoxylin-eosin stain. In addition, staining with pax 2 was performed immunohistochemically. Samples were examined with a light microscope (Olympus® Co. CX41 Tokyo, Japan). Damage to kidney tissue was evaluated using the modified scoring system. Histopathological scoring was made according to the highest area. By semi-quantitative analysis; four categories were determined (0: Absent 1: Minimal 2: Mild 3: Moderate 4: Severe) and parameters were scored accordingly.

To determine the extent of tubular damage, glomerular damage and interstitial damage, ''tubular dilatation, proteinous material accumulation in tubule, tubular epithelial cell change, glomerular damage (fibrosis/atrophy/thrombosis), interstitial fibrosis, interstitial congestion/hemorrhage, interstitial habilitate mononuclear cell infiltration'' ' parameters were used. Pax 2 expression levels were graded using the 0-3+ range. (pax 2; 0: no staining, 1: less than 10% nuclear staining of renal tubule epithelial cells, 2: nuclear staining of 10-30% of renal tubule epithelial cells, 3: nuclear staining of more than 30% of renal tubule epithelial cells staining) (11).

Malondialdehyde (MDA) levels and superoxide dismutase (SOD) and catalase (CAT) activities were measured by calculating absorbance in a spectrophotometer (Shimadzu UV 1800, Kyoto, Japan). The thiobarbituric acid test was used to calculate MDA levels (12). SOD enzyme activity was determined by Marklund et al. It was calculated according to the method reported by (13). CAT activity was measured as stated by Aebi et al. (14).

Statistical Package for the Social Sciences (22.00 SPSS Inc., Chicago, IL) was used for statistical analysis. Power analysis was used and the sample size was calculated as at least 8 for each group with 80% accuracy. Chi-square for categorical variables and independent t-test for numerical values were used. P value < 0.05 was considered statistically significant.

Results

Blood MDA levels and SOD and CAT enzyme activity levels are shown in **Table 2.** MDA levels in the amiodarone group were significantly higher than those in the amiodarone + astaxanthin group ($p < 0.05$). SOD and CAT enzyme activities were compared, the values in the amiodarone group were lower than the amiodarone + astaxanthin group, the difference was statistically significant ($p < 0.05$).

Table 2. Distribution of blood malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) levels according to groups.

Gruplar (n = 8)	MDA (nmol/mg)	SOD (U/mg)	CAT (U/mg)
Control group	8.32 ± 1.65	60.5 ± 9.61	108.4 ± 19.7
Amiodarone group	20.12 ± 4.38*	25.12 ± 5.71*	56.75 ±13.22*
Amiodarone+astaxanthin group	11.23 ± 2.27*	43.87 ± 8.54*	87.59 ± 15.23*

MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase

Data were expressed as ± standard deviation

* Significant difference between groups 2 and 3 ($p < 0.05$)

There was no difference between the groups in terms of the macroscopic appearance of the tissues. When the damage to the kidney tissue was scored, the histopathological damage in the amiodarone group was significantly higher than the amiodarone + astaxanthin group ($p < 0.05$). Damage levels in tissues are shown in **Table 3.**

Table 3. Distribution of histopathological findings according to groups

Groups (n = 10)	Hemorrhage	Fibrosis	Glomerular atrophy	Edema	Inflammatory cell infiltration
Control group	0	0	0	0	0
Amiodarone group	2*	2*	2*	2*	3*
Amiodarone+astaxanthin group	1*	1*	1*	1*	1*

* Significant difference between groups 2 and 3 ($p < 0.05$).

The highest area was determined and histopathological scoring was performed. Four categories (0: Absent 1: Minimal 2: Mild 3: Moderate 4: Severe) were determined by semi-quantitative analysis and the parameters were scored accordingly.

In the control group, the parenchyma structure in the kidney tissue appeared normal and the cellular architecture was intact **(Figure 1A).** Glomerular atrophy, inflammatory cell infiltration and interstitial fibrosis were observed in the amiodarone group **(Figure 1B).** Minimal parenchymal damage and tubular cell damage were observed in the amiodarone + astaxanthin group **(Figure 1C).**

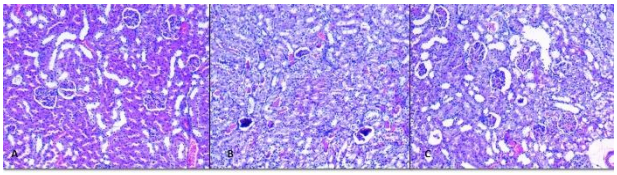

Figure 1. Evaluation of the kidney with light microscopy. (A) Renal parenchyma view of rats in the control group (H&E, x200). (B) Renal parenchyma view of rats in the amiodarone group. Significant hemorrhage, mononuclear inflammatory cell infiltration and glomerular atrophy were observed (H&E, x200). (C) Renal parenchyma view of rats in the amiodarone+astaxanthin group. Local mononuclear inflammatory cell infiltration and hemorrhage were observed (H&E, x200).

When the Pax 2 stained preparations were examined, it was determined that the parenchymal destruction caused by amiodarone was reversed with astaxanthin **(Figure 2A, B, C).**

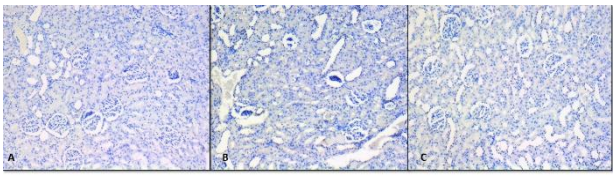

Figure 2. Evaluation of kidney with pax 2 immunostain. (A) Renal parenchyma view of rats in the control group (x200). (B) Renal parenchyma view of rats in the amiodarone group. (x200). (C) Renal parenchyma view of rats in the amiodarone+astaxanthin group. (x200**).**

Discussion

In this randomized controlled experimental study, the effect of astaxanthin on the renal toxicity of amiodarone was investigated. To our current knowledge, this is the first study to investigate the protective effect of astaxanthin against amiodarone-induced nephrotoxicity. Short-term findings show that MDA levels in the amiodarone group were significantly higher than those in the amiodarone + astaxanthin group, while SOD and CAT enzyme activities were significantly lower ($p < 0.05$).

In addition, it was observed that tissue damage, which was more pronounced in the amiodarone group, regressed with the administration of astaxanthin. Our results were consistent with the thesis that kidney damage and structural changes secondary to amiodarone could be reduced by administering an antioxidant.

Amiodarone, which is a class 3 derivative antiarrhythmic, has many side effects such as acute liver failure, nephrotoxicity, cardiac arrest, adult respiratory distress syndrome and hypotension (15). Robin et al. stated that recurrent hypotension attacks play a role in amiodarone-induced kidney damage (16). Serviddio et al. suggested that amiodarone increases mitochondrial hydrogen peroxide synthesis, resulting in increased lipid peroxidation and kidney damage (17). Other theories regarding the pathogenesis of amiodarone-induced nephrotoxicity include oxidative stress, free radical increase, phospholipase inhibition, and membrane destabilization (18).

Since oxidative stress plays an important role in the pathogenesis of amiodarone toxicity, antioxidants can be used to reduce these side effects. Astaxanthin, which is a pigment in many plants, is a natural component that contains antioxidant, antiproliferative, anti-inflammatory properties and is also called carotenoid (19). Astaxanthin, which is also obtained from algae such as Haematococcus pluvialis or fungi such as Phaffia rhodozyma and is in red, has been touted as a unique antioxidant that prevents cell and tissue damage caused by oxidative stress (10). Therefore, we thought that the astaxanthin molecule, which has strong antioxidant properties, may be effective in preventing amiodarone-induced nephrotoxicity.

Pax 2 is is localized in the long arm of 10th chromosome. Pax 2 protects the cell from cell death during cellular stress. Pax 2 gene expression has been shown to increase during oxidative stress. With this increase, the ability of the cell to be protected from cell death increases (20). We demonstrated that the parenchymal destruction caused by amiodarone was reversed with astaxanthin by using pax 2 immunohistochemical stain.

In this study, subsequent addition of astaxanthin to rats given amiodarone resulted in a decrease in MDA levels and an increase in SOD and CAT enzyme activities. The protective effect of the astaxanthin molecule has also been confirmed histopathologically. When the parameters indicating damage were scored, the score in the amiodarone+astaxanthin group was found to be lower than that in the amiodarone group. Limitations of the study are the difficulty in adapting the findings in rats to humans and the relatively small sample size.

Conclusion

As a result, astaxanthin was found to be effective in preventing amiodarone-induced kidney damage.

References

1. Nuttall SL, Routledge HC, Kendall MJ. A comparison of the beta1-selectivity of three beta1-selective beta-blockers. J Clin Pharm Ther 2003;28(3):179-186.
2. Sakr SA, El-Gamal EM. Effect of grapefruit juice on amiodarone induced nephrotoxicity in albino rats. Toxicol Ind Health 2016;32(1):68-75.
3. Somani P, Bandyopadhyay S, Klaunig JE, ve ark. Amiodarone- and desethylamiodarone-induced myelinoid inclusion bodies and toxicity in cultured rat hepatocytes. Hepatology 1990;11(1):81-92.
4. Ruch RJ, Bandyopadhyay S, Somani P, ve ark. Evaluation of amiodarone free radical toxicity in rat hepatocytes. Toxicol Lett 1991;56(1–2):117-126.
5. Pan S, Chenkai Z. Biological and neurological activities of astaxanthin (Review). Mol Med Rep 2022;26(4):300.
6. Zheng YF, Bae SH, Kwon MJ, ve ark. Inhibitory effects of astaxanthin, β-cryptoxanthin, canthaxanthin, lutein, and zeaxanthin on cytochrome P450 enzyme activities. Food Chem Toxicol 2013;59:78-85.
7. Guerin M, Huntley ME, Olaizola M: Haematococcus astax- anthin: Applications for human health and nutrition. Trends Biotechnol 2003;21:210-216.
8. Baccouche B, Benlarbi M, Barber A J. Short-term administration of astaxanthin attenuates retinal changes in diet-induced diabetic psammomys obesus. Curr Eye Res 2018;43:1177-1189.
9. Wu H, Niu H, Shao A, ve ark. Astaxanthin as a potential neuroprotective agent for neurological diseases. Mar Drugs 2015;13: 5750-5766.
10. Grimmig B, Kim SH, Nash K, ve ark. Neuroprotective mechanisms of astaxanthin: A poten- tial therapeutic role in preserving cognitive function in age and neurodegeneration. Geroscience 2017;39:19-32
11. Zhai QJ, Ozcan A, Hamilton C, ve ark. PAX-2 expression in non-neoplastic, primary neoplastic, and metastatic neoplastic tissue: A comprehensive immunohistochemical study. Appl Immunohistochem Mol Morphol 2010;18:323-32.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid rection. Anal Biochem 1979;95:351-358.
13. Marklund S, Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-474.
14. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-126.
15. Campbell N, Agarwal K, Alidoost M, ve ark. Acute Fulminant Hepatic Failure and Renal Failure Induced by Oral Amiodarone: A Case Report and Literature Review. Cureus 2020;12(5):e8311.
16. Robin P, Prerna D, Saurav S, ve ark. Acute liver and renal failure: a rare adverse effect exclusive to intravenous form of amiodarone. Case Rep Crit Care 2016;52:328.
17. Serviddio G, Bellanti F, Giudetti AM, ve ark. Mitochondrial oxidative stress and respiratory chain dysfunction account for liver toxicity during amiodarone but not dronedarone administration. Free Radic Biol Med. 2011;51(12):2234-2242.
18. Ray S, Bagchi D, Lim PM, ve ark. Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. Res Commun Mol Pathol Pharmacol 2001;109:165-197.
19. Mularczyk M, Michalak I, Marycz K. Astaxanthin and Other Nutrients from Haematococcus Pluvialis-Multifunctional Applications Mar Drugs 2020;18:459.
20. Ateş D. Overin müsinöz tümörlerinde pax 2, pax 8 ve cdx 2 ekspresyonu: metastaz ayırıcı tanısı ve patogenezdeki yeri. Hacettepe Üniversitesi Tıp Fakültesi Patoloji Anabilim Dalı Uzmanlık Tezi 2013; 15-6.