



The effect of oxaliplatin on heart tissue of the rats

Oksaliplatin'in sıçan kalp dokusu üzerindeki etkisi

Oxaliplatin and cardiac safety

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Öz

Amaç: Bu çalışmada amacımız serumda troponin I ve S100A1 düzeylerini ve kalp dokusunda oksidatif stress parametrelerini kullanarak sıçan kalbinde oksaliplatin (OXA)'nin kardiyotoksitesini araştırmaktır. Gereç ve Yöntem: 6-8 aylık sıçanlara akut OXA tedavisi (4 mg/kg/1., 3. ve 5. gün) uygulandı. Çalışmanın 7. ve 14. günlerinde kalp ve serum örnekleri elde edildi. Kronik OXA tedavisi (4 mg/kg/haftadaikigün/4 hafta) uygulandı ve çalışmanın 28. Gününde ve ilaçların tamamlanmasından sonraki 21. Gününde sıçanlar sakrifiye edilerek kalp ve serum örnekleri elde edilmiştir. Bulgular: Tüm OXA tedavi gruplarında antioksidan seviyeleri ve serum kardiyotoksiste belirteçlerinden troponin I ve S100 A1 seviyeleri kontrol grubundan istatistiksel olarak anlamlı farklı saptanmadı (p>0.05). Tartışma: OXA 4 mg/kg akut ve kronik uygulamalarıyla anlamlı bir miyokardiyal oksidatif hasar oluşturmamıştır. Oxa tedavisi kardiyotoksiste açısından güvenli gözükmektedir.

Anahtar Kelimeler

Kardio-Onkoloji; Oxaliplatin; S100A1; Troponin I

Abstract

Aim: This study investigates the cardiotoxicity of Oxaliplatin (OXA) on rat heart by using oxidative stress parameters on myocardium and troponin I and S100A1 levels of serum. Material and Method: Acute OXA treatment (4 mg/kg/1st, 3rd and 5th days) has been administered to the 6-8 months old rats. The heart and serum samples were obtained at 7th and 14th days of the study. Chronic OXA treatment was (4 mg/kg/two days of week/4 weeks) administered, rats were sacrificed and heart and serum samples were obtained at the 28th day and at the 2nd day following the completing administration of drugs. Results: The results of all OXA treatment group's antioxidant levels and serum cardiotoxicity markers; troponin I and S100 A1 levels were not significantly different compared to the control group (p>0.05). Discussion: OXA did not produce significant oxidative myocardial damage when given 4 mg/kg in acute and chronic administration. Oxa treatment seems safe in terms of cardiotoxicity.

Keywords

Cardio-Oncology; Oxaliplatin; S100A1; Troponin I

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Introduction

Chemotherapeutic agents are thought to have the potential to damage cardiac tissue. The risk and mechanism of chemotherapy-induced cardiotoxicity may vary depending on the type and severity of the anticancer regimen. Numerous chemotherapeutic drugs may cause adverse cardiovascular side effects such as arterial hypertension, heart failure, and thromboembolic events. Anthracyclines (eg., doxorubicin, epirubicin, daunorubicin, idarubicin) have been extensively studied because of the high incidence of cardiac insufficiency due to cardiotoxicity and significant cardiovascular effects [1,2]. However, cardiotoxicity may also occur with other chemotherapeutic agents including alkylating agents (cyclophosphamide, ifosfamide), platinum agents, antimetabolites (5-fluorouracil, capecitabine), antibiotics (mitoxantrone, mitomycin, bleomycin) and antimicrobial agents (taxanes) [3].

Although cardiotoxicity has been reported by oxidative stress induced by cisplatin-related reactive oxygen metabolites from platinum group chemotherapeutics, fewer studies have been reported on oxaliplatin(OXA) cardiotoxicity [4,5,6].

For this reason, in this study; we studied whether cardiotoxicity was present in the acute or chronic administration of OXA or not. If such a side effect is present, the recycling of this toxicant was assessed with biochemical markers of damage.

Material and Method

Animals and experimental design

Wistar rats, 6-8 months old albino male, were obtained from Animal Care and Research Unit of Adnan Menderes University (ADU, Aydin, Turkey) all experiments were performed according to the principles and guidelines of ADU Animal Ethical Committee's approval. This study mainly planned to assess the ototoxic effect of OXA, but the remained tissues have been evaluated on cardiotoxicity to decrease the animal number used in medical researches (HADYEK, 64583101/2014). 4 mg/kg OXA (Eloxatin®, Sanofi, U.S.) have been diluted in 5% glucose solution just before intraperitoneal (i.p.) administration. The experimental groups were as follows:

Control (n=7): The rats in this group were administered 5% glucose solution i.p.

Acute OXA treatment (n=7): Animals with OXA treatment on the 1st, 3rd and 5th days of the study, animals were sacrificed at day 7.

Acute OXA treatment + waiting period (n=7): Animals with OXA treatment on the 1st, 3rd and 5th days of the study, animals were sacrificed at day 14 to see the OXA effect one week later after drug cessation.

Chronic OXA treatment (n=7): OXA was injected two days in a week (Monday and Thursday) for 4 weeks, and the rats were sacrificed on the 28th day.

Chronic OXA treatment + waiting period (n=7): OXA was injected two days in a week (Monday and Thursday) for 4 weeks. The rats were sacrificed 21 days after the completion of the injections.

In each group at the end of the study time, the blood was withdrawn by cardiac puncture and the tissues taken out were immediately placed on ice, under the anesthesia of Ketamine and Xylazine (50 mg/kg and 5 mg/kg, respectively).

Determination of oxidant/antioxidant parameters in heart tissues

Dissected heart tissues were immediately rinsed in ice-cold phosphate-buffered saline. Tissues were homogenized (2000 rpm/min for 1 min, 1/10 w/v) using a Teflon-glass stirrer (IKA Overhead Stirrer; IKA-Werke GmbH & Co. KG, Staufen, Germany) in 10% 150 mM phosphate buffer (pH 7.4) in an ice bath. The homogenate was centrifuged (HettichZentrifugen, Mikro 200 R, Tuttlingen, Germany) at 6000 g for 10 min at 4°C. The supernatants were frozen at -80°C (Glacier Ultralow Temperature Freezer, Japan) until analyzed.

Protein concentrations in supernatants were measured by aspectrophotometer (Shimadzu UV-1601, Kyoto, Japan) using commercially available kits by the Biuret method (Archem Diagnostic Ind. Ltd., Istanbul,Turkey) and the results are expressed as mg/ml protein.

Malondialdehyde(MDA) level

The concentrations of MDA were determined according to the method of Yoshioka et al. [7]. The tissue homogenate was used for the lipid peroxidation estimation, which was applied by measuring the formation of thiobarbituric acid reactive substances (TBARS). Absorbance was measured by using a spectrophotometer at 532 nm. The concentration of MDA was calculated by the absorbance complex (absorbance coefficient $\epsilon=1.56 \times 10^5 \text{ M/cm}$) and expressed as nmol/mg tissue protein.

Total glutathione (GSH) level

The amount of GSH in supernatants were measured according to the method described by Tietze [8]. The supernatant was used to determine GSH using 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). Absorbance was spectrophotometrically determined at 412 nm. The results were determined by comparison with a standard aqueous solution of GSH (Sigma ChemicalCo., St. Louis, Missouri, USA) and expressed as mg/g tissue protein.

Catalase (CAT) activity

CAT activity was determined according to the method of Bergmeyer et al. [9], and was measured spectrophotometrically at 240 nm. The principle of the assay was based on the determination of the rate constant of hydrogenperoxide decomposition by the CAT enzyme and expressed as k/mg tissue protein.

Superoxidedismutase (SOD) activity

SOD activity was determined according to the method of Sun et al. [10], and the absorbance was measured at 560 nm by a spectrophotometer. The principle of this method is based on the inhibition of nitro blue tetrazolium reduction by the xanthine on xanthine oxidase system as a superoxide generator. SOD activity was then measured by the degree of inhibition of this reaction and the results are shown as U/mg tissue protein. All these enzyme activity assays were analyzed in duplicate, and were averaged.

Determination of Troponin and S100A1 in serum

The blood was centrifuged (HettichZentrifugen, Mikro 200 R, Tuttlingen, Germany) at 10000 g for 10 min at 4°C and the serum kept at -80°C until analyzed. Serum Troponin I levels

were determined with immunoassay on Advia Centaur CP (Siemens, Germany) autoanalyzer. Rat serum S100A1 levels were determined by using rat protein S100A1 ELISA kit (Sensitivity 1.56 ng/ml; Cusabio Biotech, China), according to the manufacturer instructions and studied on Bioctechautomatic Elisa plate reader (USA). The optical density of each well was determined within 5 minutes using a micro plate reader set to 450 nm.

Statistical analysis

Statistical package program SPSS 20 (IBM Corp. released 2011; IBM SPSS Statistics for Windows, Version 20.0, Armonk, NY: IBM Corp.) was used to evaluate the data. Variables, mean \pm standard deviation and median (Maximum-Minimum) percentage and frequency values were used. Also, the homogeneity of the variances from the preconditions of the parametric tests was checked by the "Levene" test. The assumption of normality was checked by the "Shapiro-Wilk" test. For three or more group comparisons, One-way analysis of variance and multiple comparisons of Tukey HSD test was used and if it was not provided by this test Kruskal Wallis, and Bonferroni-Dunn test of multiple comparison tests were used.

If the relationship between the two variables does not satisfy the parametric test prerequisites, the Spearman Rho Correlation Coefficient is used. Statistical significance level was accepted as $p < 0.05$.

Results

Tissue SOD, CAT, GSH and MDA levels

None of the results in all acute, acute waiting, chronic and chronic waiting OXA treatment group's SOD, CAT, GSH and MDA levels were significantly different from the control group ($p > 0.05$). These antioxidants have shown that OXA does not cause oxidative damage in our study protocol (Table 1).

Serum cardiac parameters

Troponin I and S100 A1 level

Serum cardiotoxicity markers; troponin I and S100 A1 level changes in all of the acute, acute waiting, chronic and chronic waiting OXA treatment groups were not significantly different compared to the control group ($p > 0.05$) (Table 2).

Discussion

Cardiotoxicity was investigated by using biochemical parameters. First, the (anti)oxidative status of the heart tissue has been evaluated after the exposure of OXA. Secondly, troponin

Table 2. Serum troponin I and S100A1 levels of all groups.

Groups	Troponin I (ng/ml)	S100A1 (ng/ml)
Control	1.14 \pm 0.52	10.25 \pm 8.81
Acute Oxaliplatin	1.36 \pm 0.78	5.62 \pm 2.38
Acute Oxaliplatin + waiting period	2.05 \pm 1.66	7.40 \pm 4.09
Chronic Oxaliplatin	2.12 \pm 1.39	8.31 \pm 2.19
Chronic Oxaliplatin +waiting period	5.15 \pm 4.30	6.37 \pm 2.78

I levels have been assessed as well-known markers of cardiac injury to clarify whether OXA is harmful. Thirdly, the S100A1 level of serum was investigated as a possible new marker of onco-cardiology.

The heart muscle is highly perfused, therefore it's exposure is only possible by high concentration drugs. Based on the tissue levels of SOD, CAT, GSH and MDA results of this study, the administration of OXA in these acute and chronic routes did not severely induce oxidative stress in the heart. Troponin I and S100 A1 levels in the serum as indicators of cardiac damage were also not detected in the control group, indicating that cardiotoxicity did not occur.

SOD and CAT are primary antioxidant enzymes. Hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻) and hydroxyl radicals are biologic products of reduction of oxygen (O₂) molecules; these antioxidant enzymes protect the organism against these radicals [11,12].

In an experimental animal study of the hepatic toxicity of OXA in vitro conditions by Tabassum et al., mitochondria were incubated with OXA. Similar to our study, SOD antioxidant enzyme was also evaluated. Unlike our study, SOD enzyme was found to be significantly higher than the control in the group treated with 500 μ mol OXA [13]. However, in our study, by not determining increased SOD enzyme in heart tissue in any of the acute and chronic administration groups at 4 mg/kg dose we found that OXA didn't have a cardiotoxic effect.

OXA in combination with 5-fluorouracil was found to cause cardiotoxicity due to elevated troponin I in an animal study [14]. However, as a platinum group chemotherapeutic agent, OXA is reported to have a much lower toxic effect than cisplatin [15]. However, OXA alone has not been studied for toxic effects on heart tissue. In our study, we did not detect the cardiotoxic effect of OXA in serum and tissue markers. However, not studying the cardiotoxic effect at different doses is one of the limitations of our study.

In an experimental animal study in which cardiotoxicity was assessed by platinum-based chemotherapeutics, in accordance with the literature, a significant increase in MDA levels in comparison with the control group was found while SOD and GSH were found to be significantly decreased in mice given 10 mg/kg IP cisplatin [16]. In our study, there was no significant difference between OXA and these enzymes compared to the control group.

It has been reported that S100A1 is most abundant in cardiomyocytes [17] and finds in the extracellular compartment after heart ischemia [18]. In our previous study, we have shown the disruption of S100 A1 after exposure of lapatinib or trastuzumab therapy [19]. Beside of the Troponin I and MDA levels, S100 A1 level did not give any significant changing value.

As a conclusion, OXA did not produce significant oxidative myo-

Table 1. Antioxidant and oxidant status in heart tissues of OXA treated rats in different treatment scheme.

Groups	Parameters			
	MDA (nmol/mg protein)	CAT (nmol/mg protein)	SOD (U/mg protein)	GSH (U/mg protein)
Control	2.30 \pm 0.86	38.18 \pm 8.17	5.51 \pm 1.35	1.70 \pm 0.23
Acute Oxaliplatin	1.36 \pm 1.05	40.54 \pm 9.48	4.39 \pm 0.37	1.76 \pm 0.21
Acute Oxaliplatin+ waiting period	1.52 \pm 0.37	22.29 \pm 8.47	5.70 \pm 2.89	1.83 \pm 0.32
Chronic Oxaliplatin	1.71 \pm 0.37	53.35 \pm 12.93	6.28 \pm 3.24	1.40 \pm 0.43
Chronic Oxaliplatin+ waiting period	1.44 \pm 0.23	34.48 \pm 18.83	5.42 \pm 2.08	1.74 \pm 0.22

cardial damage when given 4 mg/kg. We clearly demonstrated that OXA treatment alone did not show detrimental effect when given acute and chronic administration.

Human Rights Statement: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Animal and human rights statement

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