Protective Effect of Pycnogenol on Cisplatin Induced-Cardiotoxicity in Rats

Ufuk Eryilmaz¹, Saliha Aksun², Buket Demirci³

¹Adnan Menderes University Faculty of Medicine, Department of Cardiology, Aydın, Turkey
²Katip Çelebi University Faculty of Medicine, Department of Medical Biochemistry, İzmir, Turkey
³Adnan Menderes University Faculty of Medicine, Department of Medical Pharmacology, Aydın, Turkey

Abstract

Objective: This study investigated the cardiotoxicity of cisplatin (CIS) on rat heart by using the oxidative damage of the rat myocardium, Troponin I and serum S100A1 levels. Previous studies have reported that cell-protective effect of Pycnogenol® (PYC) depended on its antioxidant and anti-inflammatory properties. Hence, the myocardial protective effect of PYC was investigated in this study.

Materials and Methods: Rats were randomly assigned to four groups with 5 per group. The experimental groups were as follows: Control Group, Pycnogenol Group: 10 mg/kg Pycnogenol intraperitoneally for 7 days, Cisplatin Group: 15 mg/kg single injection of cisplatin on the 5th day intraperitoneally, Cisplatin + Pycnogenol Group: 10 mg/kg Pycnogenol for 7 days intraperitoneally, plus 15 mg/kg single injection of cisplatin on the 5th day. The heart and serum samples were obtained on the 8th day.

Results: CIS and PYC Co-treatment group had increased catalase level (from 43.61±15.16 to 60.80±21.36, p< 0.019) and prevented Troponin I elevation (from 7.34±6.20 to 3.03±1.36). The S100A1 level was significantly reduced by CIS (from 10.25 ±8.8 to 3.99 ± 2.87, p< 0.035) and was restored by PYC treatment (32.07±29.23).

Conclusion: Injured cardiomyocytes released Troponin I after exposure to CIS and PYC, which can protect the cells from CIS cardiotoxicity, increased the tissue catalase level. Additionally, PYC treatment increased serum level of S100A1.

Keywords
Cardio-oncology, Pycnogenol, S100A1, Troponin I

Received/Geliş Tarihi : 31.07.2017
Accepted/Kabul Tarihi : 24.09.2017

doi:10.4274/meandros.85856

Address for Correspondence/Yazışma Adresi:
Ufuk Eryilmaz MD,
Department of Cardiology, Medical Faculty,
Adnan Menderes University, Aydın
Phone: +90 535 981 97 12
E-mail: drufukeryilmaz@gmail.com

©Meandros Medical and Dental Journal. Published by Galenos Publishing House.
Introduction

Cisplatin (CIS) is one of the most commonly used alkylating chemotherapeutic agents. The US Food and Drug Administration (FDA) had approved the drug for the first time in 1978 for the treatment of ovarian and testicular cancers (1). Today, it is used for the treatment of lung, head and neck, germ cell tumors and lymphomas. Nephrotoxicity, neurotoxicity, ototoxicity, and cardiotoxicity are the known side effects of the drug (2). The most significant factors limiting its utilization are nephrotoxicity and cardiotoxicity, that can occur acutely or cumulatively and by similar mechanisms (3).

Pycnogenol (PYC) is the US-registered name of the product obtained from the shell of the pine tree (Pinus pinaster) and includes water-soluble bioflavonoids. Pycnogenol is used for a wide variety of medical conditions caused by oxidative stress such as nephrotoxicity, diabetes, hepatotoxicity, and tinnitus (4-6). It was shown to protect against daunorubicin-induced cardiotoxicity by its antioxidant effect (7). However, it is not known whether PYC is protective against CIS cardiotoxicity.

One-way ANOVA was used for three or more group comparisons and the Tukey HSD test was used for multiple comparison tests. If the preconditions are not met, Kruskal Wallis and the Bonferroni-Dunn test from multiple comparison tests are used. Correlation analysis between two continuous variables was also used.

Cardiac injury has been evaluated by oxidative damage on CIS-induced heart tissue. While troponin I is a well-known marker of cardiac injury, S100A1 originates in cardiomyocytes (8) and it is not clear whether its expression correlates with troponin or can be useful as a new marker of cardio-oncology.

Several studies have been performed about the cardio protective effects of natural products and found as promising during the cancer chemotherapy (7,9). On the other hand, the natural product can behave as an adjuvant and might further increase the toxicity of antineoplastic medicine (10). Therefore, the aim of the study was to investigate safety and efficacy of PYC on CIS induced cardiotoxicity.

Material and Methods

Animals and experimental design

4-6 months old male Wistar albino rats were obtained from the 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 evaluated the remained heart tissue of another study about “the protective effects of Pycnogenol® on cisplatin ototoxicity” after taking the new approval from the Committee (64583101/2013/037) to decrease the animal number used in medical experiments. The experimental groups were as follows:

- **Control Group:** The rats in this group were administered physiologic serum for seven days intraperitoneally and served as the healthy animal group.

- **Pycnogenol Group:** Healthy animals with 10 mg/kg Pycnogenol (gifted from Horpag Research Ltd Geneva, Switzerland) treatment intraperitoneally for seven days, from the first day of the study and the subsequent days. The Pycnogenol dose was decided with a nephrotoxicity research (5).

- **Cisplatin Group:** The rats in this group were administered 15 mg/kg single injection of cisplatin (Platinol®, Bristol-Myers Squibb, Istanbul, Turkey) intraperitoneally on the 5th day of study.

- **Cisplatin + Pycnogenol Group:** The rats in this group were administered 10 mg/kg Pycnogenol treatment intraperitoneally for seven days, from the first day of the study and the subsequent days. Additionally, on the 5th day of the study, 15 mg/kg single injection of cisplatin was given.

On the 8th day of study, under the anesthesia of Ketamine and Xylazine (50 mg/kg and 5 mg/kg, respectively), the blood was withdrawn by
cardiac puncture, and the hearts were taken out immediately.

**Determination of oxidant/antioxidant parameters in heart tissues**

**Preparation of tissue samples:**
Rat hearts were homogenized for 10 minutes at 8,000 rpm using a homogenizer over ice after weighing and cold chaining in a 50 mM phosphate tamponade (pH=7.0) (Heidolph, Silentcruster M, Germany). After homogenization, they were centrifuged with a refrigerator at +4 °C for 10 minutes and supernatants were kept at -80 °C (Hermle, Z 400 K, Germany). Tissue homogenate protein results were obtained as mg/dl with Abbott urinary protein kit using an autoanalyzer (Architect, Abbott).

**Catalase (CAT) activity**
Aebi method based on kinetic measurement was used for diagnosis of CAT (11). It is measured by the reaction which gives rise to water and oxygen from hydrogen peroxide $\text{H}_2\text{O}_2$.

\[
\text{2 H}_2\text{O}_2 \xrightarrow{\text{catalase}} 2 \text{H}_2\text{O} + \text{O}_2
\]

In this study, CAT activity was detected as a decrease in $\text{H}_2\text{O}_2$ concentration in a time at 240 nm in a spectrophotometric (Dynamica Halo DB-20s, UK) analysis. Homogenates were diluted to a 1:5 dilution with 50 mM phosphate tamponade (pH 7.0). 1.00 ml $\text{H}_2\text{O}_2$ solution (30 mM) was added to 2.00 mL homogenate, and absorbance changes were recorded at 240 nm with 15-second intervals. Same procedures were reduplicated with randomized samples and activity, baseline and 30-second absorbances were calculated. Activity unit was reported as a ratio of obtained values to tissue protein level in k/mg.

**Determination of Troponin and S100A1 in serum**
The blood was centrifuged (Hettich Zentrifugen, Mikro 200 R, Tuttlingen, Germany) at 10,000 rpm for 10 min at 4 °C and the serum kept at -80 °C until the time of analysis. Serum Troponin I level was determined with immunoassay on Advia Centaur CP (Siemens, Germany) autoanalyzer. Rat serum S100A1 levels were determined by using rat protein S100A1 ELISA kit Cusabio Biotech, China), according to the manufacturer instructions and studied on automatic Elisa plate reader (Biotech ELx800, USA). The optical density of each well was determined within 5 minutes using a micro plate reader set to 450 nm.

**Statistical analysis**
The results of tests were expressed as the number of observations (n), mean ± standard deviation, median and %25-%75 percentage values. The results of the homogeneity (Levene’s Test) and normality tests (Shapiro Wilk) were used to decide which statistical methods to apply in the comparison of the study groups. Normally distributed and with homogeneous variances groups were compared independent three or more groups by Analysis of Variance. Multiple comparison tests, the Tukey HSD test was used. According to those tests results parametric test assumptions were not available for some variables, so the comparisons of three independent groups were performed by Kruskal Wallis test. Multiple comparison tests, the adjusted Bonferroni-Dunn test was used. If the relationship between the two variables does not satisfy the parametric test prerequisites, the Spearman Rho correlation coefficient is used. Data were analyzed using SPSS 20 (IBM Corp. Released 2011; IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) software. P< 0.05 was used for statistical significance.

**Results**

**CAT level in the Heart Tissue**
PYC did not affect CAT activity on healthy tissue, and 15 mg/kg of CIS did not reduce catalase level when compared to the control group, but co-treatment of PYC and CIS significantly triggered the antioxidant catalase level up to 60.80± 21.37 (Table 1).

**Serum Troponin I and S100 A1 level**
Serum Troponin I level of CIS group was found elevated in the serum, and PYC was found to prevent the elevation. S100A1 level highly increased after PYC treatment (Table 1). Heart tissue catalase level showed a positive correlation with serum S100A1 level; $r=0.47$ $p=0.04$. One is increasing, while the other is seen as an increasingly “moderate” statistically significant relationship (Figure 1).

**Discussion**
In our study, it was found that PYC did not have any effect on CAT activity in healthy tissues and cisplatin did not lead to the reduction of the catalase level. However, when PYC and CIS were used together, they were found to trigger the antioxidant catalase level.
The S100A1 value, which was found to be positively correlated with the catalase level in the heart tissue, also increased following PYC treatment. As expected, serum Troponin I level was found elevated in CIS group, and when Pycnogenol was added to the treatment, this value was reduced due to the positive effect of Pycnogenol.

It might be expected that the adverse effects of drugs can be seen mostly in elimination organs such as liver and kidney. Moreover, the heart tissue is highly perfused; therefore, exposures to the drugs occur in high concentration. Due to technical problems, we were unable to assess the oxidant status such as the status of malondialdehyde (MDA) in the heart; however, El-Awady et al. (12) reported that 10 mg/kg single dose of CIS significantly increased MDA level of heart tissue and proved that CIS is harmful to the heart tissue in animal study. Based on the tissue levels of CAT found in this study, the administration of CIS in the dose of 15 mg/kg was considered not to consume the CAT activity of the heart tissue severely; only Troponin I level increased significantly as an early marker for cardiac injury. This increment was partially protected by PYC treatment together with increased CAT activity.

In addition to its antioxidant, anti-inflammatory, and antiplatelet effects, PYC was also shown to reduce oxidative stress and improve endothelial

### Table 1. Catalase activity in heart tissue and Troponin I and S100A1 levels in the serum of all experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Median (25%-75% Quartile)</th>
<th>Median (25%-75% Quartile)</th>
<th>Median (25%-75% Quartile)</th>
<th>Median (25%-75% Quartile)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td>1.14 (0.78-1.57)</td>
</tr>
<tr>
<td><strong>Cisplatin (15 mg/kg)</strong></td>
<td>2.19 (0.78-1.57)</td>
<td>5.67 (2.09-2.78)</td>
<td>7.46 (4.23-6.24)</td>
<td></td>
</tr>
<tr>
<td><strong>Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg)</strong></td>
<td>2.51 (1.44-2.78)</td>
<td>4.31 (0.96-1.33)</td>
<td>3.94 (2.09-2.78)</td>
<td></td>
</tr>
<tr>
<td><strong>Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg) + Pycnogenol (5 mg/kg)</strong></td>
<td>3.02 (1.44-2.78)</td>
<td>4.31 (0.96-1.33)</td>
<td>3.94 (2.09-2.78)</td>
<td></td>
</tr>
</tbody>
</table>

- **S100A1**
  - Control: 10.25 (3-20.78)
  - Cisplatin (15 mg/kg): 5.67 (0.01-13.68)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg): 3.02 (1.44-2.78)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg) + Pycnogenol (5 mg/kg): 2.84 (1.87-4.37)

- **Troponin I**
  - Control: 0.01 (0.96-2.78)
  - Cisplatin (15 mg/kg): 4.31 (2.09-2.78)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg): 3.94 (2.09-2.78)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg) + Pycnogenol (5 mg/kg): 2.84 (1.87-4.37)

- **CAT**
  - Control: 38.18 (31.18-46.96)
  - Cisplatin (15 mg/kg): 31.18 (31.18-46.96)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg): 34.19 (28.68-35.74)
  - Cisplatin (15 mg/kg) + Pycnogenol (10 mg/kg) + Pycnogenol (5 mg/kg): 32.67 (29.24-35.74)

*p<0.05, †Kruskal Wallis test, ‡One-Way ANOVA, ≠ Different From Control Group (Bonferroni-Dunn Test), © Different From Control Group (Tukey HSD test)

**SD:** Standard deviation

*SD: Standard deviation

**Table 1.** Correlation graph between the heart catalase activity and S100A1 level; r=0.47 p=0.04

**Figure 1.** Correlation graph between the heart catalase activity and S100A1 level; r=0.47 p=0.04

**CAT:** Catalase
function in coronary artery disease (13). Feng et al. (14) investigated the effect of PYC on cardiotoxicity in mice treated with antineoplastic drugs. In their study with the experimental mouse - doxorubicin model, they showed that Pycnogenol did not antagonize the effect of the antineoplastic agent. When administered at 150 mg/ and 200 mg/kg orally, Pycnogenol was found to prevent doxorubicin cardiotoxicity by inhibiting the elevation of creatine phosphokinase in the serum (14). The results related to levels of serum catalase and Troponin I obtained in our rat model with cisplatin support this study. The cisplatin-related cardiac dysfunction is due to the disruption of the mitochondrial membrane as well as the ultrastructural abnormalities seen in mitochondria. It was shown that, following CIS treatment, the endoplasmic reticulum stress response and apoptosis were increased in cardiomyocytes (15). CIS was shown to lead to renal injury by triggering the formation of mitochondrial reactive oxygen species in renal tubular cells (16). Since toxicity occurs with a similar mechanism, the antioxidant treatment targeting mitochondria was reported to be protective against CIS-related cardiotoxicity and nephrotoxicity (17).

On the other hand, it was reported that S100A1 is most abundant in cardiomyocytes (8) and it is found in the extracellular compartment after heart ischemia (18). The other member of S100 family, S100B protein expression was suggested as a new forensic marker for cocaine-induced heart injury (19). The crucial role of S100A1 for cardiac performance and contractility was reported (20,21). In our study, reduced release of S100A1 from injured cardiomyocytes to serum after CIS exposure was demonstrated, which was concordant with our previous study (22). Lapatinib and Trastuzumab decreased S100A1 expression in a ratio of %75. In our study, we did not report any correlation between S100A1 and Troponin I. Moreover, a positive correlation was found between S100A1 and CAT activity. A raised level of S100A1 due to theconcomitant application of PYC and CIS may give rise to the hypothesis of the beneficial effect of PYC together with anticancer drugs for decreasing cardiotoxicity. Studies using PYC to prevent cardiotoxicity in patients using cardiotoxic oncologic agents may be important in the future.

As a conclusion, a single dose of 15 mg/kg of CIS was enough to produce myocardial damage, as proven by Troponin I level in this experimental rat model. We demonstrated that PYC treatment partially prevented the detrimental effect by increasing CAT activity, which is in correlation with S100A1.

Acknowledgement
We wish to thank Horpag Research Ltd. (Geneva, Switzerland) for generously gifting us with Pycnogenol®.

Declaration of interest
The research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References


