Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Effect of pimobendan on cytokine levels in doxorubicin-induced cardiotoxicity

Burak Dik[1,a], Orhan Çorum[2,b], Ayşe Er[1*c]

[1]Selcuk University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Konya, Turkey
[2]Kastamonu University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology, Kastamonu, Turkey
Received:13.11.2018, Accepted: 15.01.2019
*aer@selcuk.edu.tr
[a]ORCID: 0000-0003-2738-6911, [b]ORCID: 0000-0003-3168-2510, [c]ORCID: 0000-0002-6900-0055

Doksorubisinle indüklenen kardiyotoksisitede pimobendanın sitokin düzeylerine etkisi

Eurasian J Vet Sci, 2019, 35, 1, 11-14
DOI: 10.15312/EurasianJVetSci.2019.216

Öz

Amaç: Doksorubisin (DOX) çeşitli insan kanserlerini tedavi etmede kullanılır, ancak kardiyotoksisitesi ve kalp yetmezliği etkileri nedeniyle kullanımı sınırlıdır. Pimobendan (PIMO) kalp yetmezliğini tedavi etmek için kullanılır. PIMO'nun proinflamatuar sitokinleri baskıladığı bildirilmesine rağmen, PIMO'nun kardiyotoksisitede antiinflamatuar sitokinler üzerindeki etkisi araştırılmadı. Bu çalışmanın amacı, PIMO'nun DOX ile indüklenen kardiyotoksisitede tümör nekrozis faktör-alfa (TNF-α), interlökin (IL)-6 ve IL-10 üzerindeki etkilerini belirlemektir.

Gereç ve Yöntem: 54 adet erkek Swiss fare Kontrol (n:6), DOX (n:24) ve DOX+PIMO (n:24) olarak gruplandırıldı. Kontrol grubuna intraperitoneal (İP) ve gavaj yöntemiyle fizyolojik tuzlu su verildi. DOX grubuna IP olarak tek doz 18 mg / kg DOX ve DOX+PIMO grubuna IP olarak tek doz 18 mg / kg DOX ve gavaj yoluyla 5 gün boyunca 1 mg / kg PIMO uygulandı. DOX uygulamasından 2, 24, 72 ve 120 saat sonra genel anestezi altında kalpten kan örnekleri alındı. Sitokin seviyeleri ELISA yöntemiyle ölçüldü.

Bulgular: Bu çalışma, DOX ile ilişkili kardiyotoksisiteye bağlı olarak proinflamatuar sitokinlerde artma ve antiinflamatuvar sitokinlerde azalma olduğunu gösterdi. PIMO uygulaması, TNF-α ve IL-6 artışını veya IL-10'un azalmasını engellemedi. Daha önce yapılan çalışmalar, kardiyotoksisitede PIMO'nun proinflamatuar sitokinler üzerindeki etkisine odaklanmıştır, ancak hiçbir çalışma PIMO'nun IL-10 düzeyindeki etkisini değerlendirmemiştir. İlginç bir şekilde, bu çalışmada, PIMO IL-10 seviyelerini arttırmadı.

Öneri: PIMO'nun DOX ile indüklenen kardiyotoksisiteye karşı koruyucu bir etkisi olmadığı ifade edilebilir.

Anahtar kelimeler: Kardiyotoksisite, sitokin, doksorubisin, pimobendan.

Abstract

Aim: Doxorubicin (DOX) is used to treat various human cancers, but its use is limited due to its cardiotoxicity and heart failure effects. Pimobendan (PIMO) is used to treat heart failure. Although PIMO has been reported to suppress proinflammatory cytokines no studies have investigated the effect of PIMO on antiinflammatory cytokines in cardiotoxicity. The purpose of this study was to determine the effects of PIMO on tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6 and IL-10 in the DOX-induced cardiotoxicity.

Materials and Methods: The 54 male Swiss mice were categorized as Control (n:6), DOX (n:24), and DOX+PIMO (n:24). The Control group received physiological saline solution intraperitoneally (IP) and gavage. DOX group received a single IP injection of 18 mg/kg DOX, and DOX+PIMO group received a single IP injection of 18 mg/kg DOX + 1 mg/kg PIMO SID for 5 days by gavage. Blood samples were collected from the heart by cardiac puncture under general anesthesia at 2, 24, 72 and 120 h following DOX administration. Cytokines levels were measured using ELISA.

Results: This study showed an increase in proinflammatory cytokines and a decrease in antiinflammatory cytokines, dependent on DOX-induced cardiotoxicity. PIMO administration did not prevent TNF-α and IL-6 increase or IL-10 decrease. Previous studies have focused on the effect of PIMO on proinflammatory cytokines in cardiotoxicity, but no study has evaluated the effect of PIMO on IL-10 level. Interestingly, in the present study, PIMO did not increase IL-10 levels.

Conclusion: It may be stated that PIMO has no protective affect against DOX-induced cardiotoxicity.

Keywords: Cardiotoxicity, cytokine, doxorubicin, pimobendan.

Introduction

Heart failure is one of the most prominent cardiac specific effects of anthracyclines (Scherrer-Crosbie 2016). Cardiac output inadequacy in meeting the organism's metabolic demands is defined as heart failure (Anderson 2000). Heart disease that develops after cancer treatment is one of the leading causes of mortality among cancer survivors (Ghigo et al 2016).

Anthracyclines are used to treat several types of cancer. These drugs are listed in main drug list on the World Health Organization (WHO). There is great interest in the cardiotoxic mechanisms of anthracyclines, which remain unknown and is likely to be multifactorial (McGowan et al 2017). Over-produced reactive oxygen species and impaired iron metabolism have been reported as major mechanisms of anthracycline cardiotoxicity. Moreover, using antioxidants and iron chelators are not beneficial in small clinical trials, which suggest that the redox cycle is not the only cause of doxorubicin (DOX)-induced cardiotoxicity (Ghigo et al 2016). Side effects of DOX and other chemotherapeutic drugs may be minimized by decreasing the production of inflammatory mediators though the use of inhibitors (Wong et al 2013).

DOX, daunorubicin, epirubicin and idarubicin are the four most common anthracyclines. In clinical practice, DOX and daunorubicin have been firstly used (McGowan et al 2017). Doxorubicin is widely used a chemotherapeutic agent over 30 years (Tacar et al 2013) in treatment of variety human cancers, including hematological malignancies and solid tumors (Ghigo et al 2016).

The most exciting works in the field of inflammatory modulation of heart failure have started with the discovery of the anti-inflammatory activity of drugs already used in the therapy of heart failure, especially the phosphodiesterase inhibitors (Anderson 2000). Pimobendan (PIMO), a phosphodiesterase 3 inhibitor, is a benzimidazole-pyridazinone derivative approved by the US Food and Drug Administration for treatment of congestive heart failure in dogs (Boyle and Leech 2012). It increases quality of life and reduces the rate of morbid events in patients with heart failure (Matsumori 2008).

Cytokines are an important factor in the pathogenesis of heart failure (Matsumori 2008, Mehra et al 2005). While blood TNF-α and IL-6 levels increase in patients with heart failure, IL-10 levels decrease (Batista et al 2009, Pecoraro et al 2016). TNF-α and IL-6 are proinflammatory cytokines, which exert a negative inotropic effect (Mehra et al 2005). On the contrary, IL-10 is an antiinflammatory cytokine that decreases proinflammatory cytokine levels (Driessler et al 2004). This may be the basis for the therapeutic effect of IL-10 (Nakano et al 2001). DOX increases TNF-α and IL-6 levels, while it decreases IL-10 level in cardiotoxicity (Ghigo et al 2016, Pecoraro et al 2016). PIMO decreases proinflammatory cytokines and exerts a positive inotropic effect (Fuentes 2004).

Although PIMO has been reported to suppress proinflammatory cytokines (Iwasaki et al 1999, Wang et al 2015), no studies have investigated the effect of PIMO on antiinflammatory cytokines in cardiotoxicity. In the present study, we aimed to demonstrate the effects of PIMO on TNF-α and IL-6 cytokine levels, mainly serum IL-10, and in DOX-induced cardiotoxicity.

Materials and Methods

Animals

In the present study, 54 male Swiss mice (8–10 weeks old, 30–35 g) were used. The study protocol was approved by the Ethics Committee of the Experimental Medicine Research and Application Centre of Selcuk University (SUDAM, 2016/20). During the experimental period, the mice were housed in standard mice cages and allowed water and food ad libitum. The mice were caged in a central facility under controlled conditions (12 h light/dark cycle and room temperature of 20 ± 2°C) at SUDAM.

Experimental procedure

The 54 mice were categorized into three groups as follows: Control, DOX, and DOX + PIMO. The Control group (n=6) received physiological saline solution intraperitoneally (IP) injection and gavage administration of once a day (SID) for 5 days; DOX group (n=24) received IP injections of 18 mg/kg (Zhu et al 2010) DOX (Adriblastina vial, Deva, Istanbul, Turkey) single dose; and DOX+PIMO group (n=24) simultaneously received IP injections of 18 mg/kg DOX single dose + gavage of 1 mg/kg PIMO (Iwasaki et al 1999) (Vetmedin tablet, Boehringer Ingelheim, Istanbul, Turkey) SID for 5 days. Blood samples were collected from the heart via cardiac puncture under general anesthesia (40 mg/kg sodium thiopental, IP, Pental sodium, IE Ulagay, Istanbul, Turkey) at 2, 24, 72 and 120 h after DOX administration and control animal. After the blood samples were taken, the mice were euthanized by cervical dislocation under general anesthesia and samples were stored at −80°C.

Cytokine measurements

The levels of TNF-α (Mouse TNFα Affymetrix eBioscience BMS607/3, Affymetrix eBioscience, San Diego, USA), IL-6 (Mouse IL-6 Affymetrix eBioscience BMS603/2, Affymetrix eBioscience, San Diego, USA) and IL-10 (Mouse IL-10 Affymetrix eBioscience BMS614/2, Affymetrix eBioscience, San Diego, USA) were measured using enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

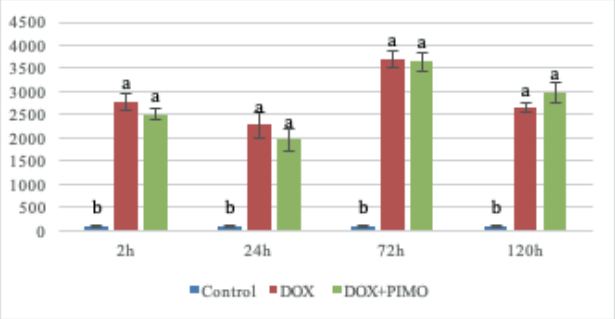

Figure 1. Effect of DOX and DOX+PIMO administrations on TNFα (pg/mL) level

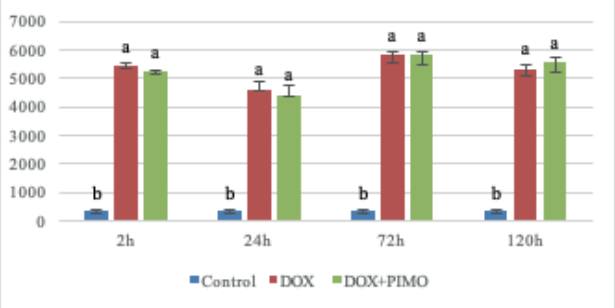

Figure 2. Effect of DOX and DOX+PIMO administrations on IL-6 (pg/mL) level

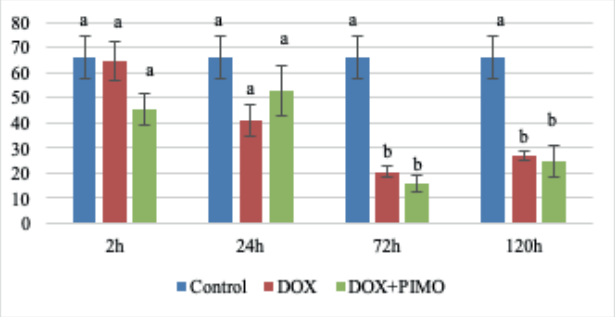

Figure 3. Effect of DOX and DOX+PIMO administrations on IL-10 (pg/mL) level

Statistical analyses

Results are presented as the mean ± standard error. TNF-α, IL-6 and IL-10 levels of each sampling time were analyzed using ANOVA and Duncan's post-hoc test (SPSS 22.0). $P < 0.05$ was considered to be statistically significant.

Results

Serum levels of TNF-α, IL-6 and IL-10 are shown in Figures 1, 2, 3, respectively. Significantly increases in TNF-α and IL-6 levels were observed following single dose DOX-induced cardiotoxicity ($P < 0.05$). The application of PIMO for 5 days could not prevent increases in TNF-α and IL-6 levels, but it caused non-significant reductions during in the first 24 h (Figures 1 and 2). DOX caused a decrease in IL-10 level at all sampling times. These decreases were significant ($P < 0.05$) at 72 and 120 h and could not be prevented by PIMO (Figure 3).

Discussion

Cardiotoxicity, defined as "toxicity that affects the heart", is a very broad condition including several phenomena, such as myocardial ischemia or infarction, cardiac dysfunction and heart failure, pericardial disease, valvular abnormalities, hypertension, and arrhythmias in the case of cancer treatments (Scherrer-Crosbie 2016).

Because inflammation affects the pathogenesis of heart failure (Scully et al 2010), researchers in the fields of immunology and immunopathology over the past several years cause to perception as an "inflammatory disease" of heart failure in part (Anderson 2000). Patients with heart failure exhibit increased TNF-α and IL-6 levels. IL-6 is reportedly a strong prognostic indicator of mortality and morbidity in patients with heart failure and acute myocardial infarction (Blum 2009). In the present study, DOX administration increased TNF-α and IL-6 levels (Figure 1 and Figure 2), which are known to be increased in cardiotoxicity. This status has been reported by several previous studies in which DOX is used at different doses (Bagalkot et al 2009, Khafaga and El-Sayed 2018, Matouk et al 2013, Mohamed et al 2004). Treatment of DOX-induced cardiotoxicity using different agents has been shown to decrease TNF-α levels and cardiotoxicity (Abdel-Daim et al 2017, Khafaga and El-Sayed 2018, Matouk et al 2013, Mohamed et al 2004). In the present study, PIMO did not prevent increases in TNF-α and IL-6 levels, but it caused a non-significant decrease at 2 and 24 h. PIMO inhibits the production of TNF-α and IL-6 (Iwasaki et al 1999, Wang et al 2015).

In patients with heart failure, IL-10 levels decrease (Batista et al 2009). When myocardial ischemia/reperfusion injury was induced in mice with IL-10 deficiency, a 75% mortality rate was observed. No deaths were observed among control mice (Yang et al 2000). Reportedly, treatment with recombinant IL-10, which is important in the recovery of cardiac cell damage in patients with a high ratio of TNF-α/IL-10, may be beneficial (Stumpf et al 2003). Interestingly, PIMO did not prevent the decline of IL-10 level (Figure 3). Although it has been reported that IL-10 decreases proinflammatory cytokine levels (Driessler et al 2004), no previous studies have investigated the effect of PIMO on cardiotoxicity-related IL-10. The results of the present study could have been by the high dose of doxorubicin and the insufficient dose of PIMO.

Conclusion

In conclusion, the use of DOX increased proinflammatory cytokine levels and decreased antiinflammatory cytokine levels in the present study. The simultaneous administrations of DOX and PIMO did not prevent decrease in IL-10 levels and increase in TNF-α and IL-6 levels. These results may depend on the dose of PIMO and further studies should been inves-

tigated the effect of PIMO on IL-10 levels by increasing its dose.

Acknowledgements

The summary of the study was presented as an oral presentation in the International Science and Academic Congress18 (INSAC18), 08-12.2018-09.12.2018, Konya Turkey, and published in the Congress Book.

References

Abdel-Daim, MM, Kilany OE, Khalifa HA, Ahmed AAM, 2017. Allicin ameliorates doxorubicin-induced cardiotoxicity in rats via suppression of oxidative stress, inflammation and apoptosis. Cancer Chemother Pharmacol 80,745-753.

Anderson, MR, 2000. The systemic inflammatory response in heart failure. Prog Pediatr Cardiol., 11,219-230.

Bagalkot V, Lee IH, Yu MK, Lee E, Park S, Lee JH, Jon S. 2009. A combined chemoimmunotherapy approach using a plasmid-doxorubicin complex. Mol Pharm., 6,1019-1028.

Batista ML, Lopes RD, Seelaender MC, Lopes AC, 2009. Anti-inflammatory effect of physical training in heart failure: role of TNF-alpha and IL-10. Arq Bras Cardiol., 93,643-651.

Blum A, 2009. Heart failure--new insights. Isr Med Assoc, J 11,105-111.

Boyle KL, Leech E, 2012. A review of the pharmacology and clinical uses of pimobendan. J Vet Emerg Crit Care (San Antonio), 22, 398-408.

Driessler F, Venstrom K, Sabat R, Asadullah K, Schottelius AJ, 2004. Molecular mechanisms of interleukin-10-mediated inhibition of NF-kappaB activity: a role for p50. Clin Exp Immunol., 135,64-73.

Fuentes VL, 2004. Use of pimobendan in the management of heart failure. Vet Clin North Am Small Anim Pract., 34,1145-1155.

Ghigo A, Li M, Hirsch E, 2016. New signal transduction paradigms in anthracycline-induced cardiotoxicity. Biochim Biophys Acta 1863,1916-1925.

Iwasaki A, Matsumori A, Yamada T, Shioi T, Wang W, Ono K, Nishio R, Okada M, Sasayama S, 1999. Pimobendan inhibits the production of proinflammatory cytokines and gene expression of inducible nitric oxide synthase in a murine model of viral myocarditis. J Am Coll Cardiol., 33,1400-1407.

Khafaga AF, El-Sayed YS, 2018. All-trans-retinoic acid ameliorates doxorubicin-induced cardiotoxicity: in vivo potential involvement of oxidative stress, inflammation, and apoptosis via caspase-3 and p53 down-expression. Naunyn Schmiedebergs Arch Pharmacol., 391,59-70.

Matouk AI, Taye A, Heeba GH, El-Moselhy MA, 2013. Quercetin augments the protective effect of losartan against chronic doxorubicin cardiotoxicity in rats. Environ Toxicol Pharmacol., 36,443-450.

Matsumori A, 2008. Role of inflammation in heart failure. Int J Cardio., 125,1–37.

McGowan JV, Chung R, Maulik A, Piotrowska I, Walker JM, Yellon DM, 2017. Anthracycline chemotherapy and cardiotoxicity. Cardiovasc Drugs Ther., 31,63-75.

Mehra VC, Ramgolam VS, Bender JR, 2005. Cytokines and cardiovascular disease. J Leukoc Biol., 78,805-818.

Mohamed HE, Asker ME, Ali SI, el-Fattah TM, 2004. Protection against doxorubicin cardiomyopathy in rats: role of phosphodiesterase inhibitors type 4. J Pharm Pharmacol., 56,757-768.

Nakano A, Matsumori A, Kawamoto S, Tahara H, Yamato E, Sasayama S, Miyazaki JI, 2001. Cytokine gene therapy for myocarditis by in vivo electroporation. Hum Gene Ther., 12,1289-1297.

Pecoraro M, Del Pizzo M, Marzocco S, Sorrentino R, Ciccarelli M, Iaccarino G, Pinto A, Popolo A, 2016. Inflammatory mediators in a short-time mouse model of doxorubicin-induced cardiotoxicity. Toxicol Appl Pharmacol., 293,44-52.

Scherrer-Crosbie M, 2016. Cardiotoxicity of oncologic treatments. Is there a uniform definition?. In: Anticancer treatments and cardiotoxicity mechanisms, diagnostic, and therapeutic interventions, Ed; Lancellotti P, Zamorano J, Galderisi M, editors.. 1st ed. London: Elsevier; pp; 35-38.

Scully R, Miller A, Grant Y, Lipshultz SE, 2010. Anthracycline, Herceptin, and CV Toxicity. In Cardiovascular Toxicology, 6,413-427.

Stumpf C, Lehner C, Yilmaz A, Daniel WG, Garlichs CD, 2003. Decrease of serum levels of the anti-inflammatory cytokine interleukin-10 in patients with advanced chronic heart failure. Clin Sci (Lond), 105,45-50.

Tacar O, Sriamornsak P, Dass CR, 2013. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol., 65,157-170.

Wang Q, Yokoo H, Takashina M, Sakata K, Ohashi W, Abedelzaher LA, Imaizumi T, Sakamoto T, Hattori K, Matsuda N, Hattori Y, 2015. Anti-Inflammatory Profile of Levosimendan in Cecal Ligation-Induced Septic Mice and in Lipopolysaccharide-Stimulated Macrophages. Crit Care Med., 43, 508-520.

Wong J, Smith LB, Magun EA, Engstrom T, Kelley-Howard K, Jandhyala DM, Thorpe CM, Magun BE, Wood LJ, 2013. Small molecule kinase inhibitors block the ZAK-dependent inflammatory effects of doxorubicin. Cancer Biol., Ther 14: 56-63.

Yang Z, Zingarelli B, Szabó C, 2000. Crucial role of endogenous interleukin-10 production in myocardial ischemia/reperfusion injury. Circulation, 101, 1019-1026.

Zhu J, Zhang J, Xiang D, Zhang Z, Zhang L, Wu M, Zhu S, Zhang R, Han W, 2010. Recombinant human interleukin-1 receptor antagonist protects mice against acute doxorubicin-induced cardiotoxicity. Eur J Pharmacol., 643, 247-253.