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Investigation of possible cardiac side effects of diclofenac in exercise-treated rats.

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Abstract

In this study, it was aimed to determine the effect of diclofenac administration on serum cardiac damage markers in exercised and non-exercised rats. In the study, 28 rats were divided into 4 equal groups. No application was made to control group, while the rats in the exercise group were floated for 30 min in a day. Diclofenac group were received at the dose of 13.5 mg/kg (intraperitoneally) diclofenac once a day, whereas the rats in the Diclofenac+Exercise group were floated for 30 min after than 1 h of administration of diclofenac at the dose of 13.5 mg/kg (intraperitoneally) once a day. At the end of one week, blood samples were taken from by cardiac puncture under general anesthesia and afterwards, rats are immediately euthanized. Rat-specific cardiac troponin I, creatine kinase-MB, lactate dehydrogenase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase, blood urea nitrogen and creatinine levels were determined in the samples. Cardiac troponin I, lactate dehydrogenase and aspartate aminotransferase levels of Diclofenac group were statistically significantly higher (P<0.05) than the all other groups, however, statistically significant (P<0.05) fluctuations were in the creatinine levels in the experimental groups. In conclusion, it is stated that the diclofenac application may cause heart damage and diclofenac may not increase heart damage when exercised together with ingestion.

Keywords: Diclofenac, Exercise, Cardiac damage.

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Introduction

Non-steroidal Anti-Inflammatory Drug (NSAID) has anti-inflammatory, antipyretic, and analgesic effects, and these effects of NSAIDs are explained by the inhibition of prostaglandins via inhibiting of Cyclooxygenase Enzymes (COX). COX is mainly divided into two forms as COX1 and COX2. COX-1 plays role in the normal physiological functions, whereas COX-2 may intensely increase in the tissue damage or inflammatory situations. NSAIDs have different selective inhibitory effects on the COX2/COX1. Selective effect of diclofenac on the COX2/COX1 is 4/1 [1]. Diclofenac has the most prescribed NSAIDs in the human and veterinary medicine because of its potent anti-inflammatory, antipyretic and analgesic effect when compared to other NSAIDs [2-4]. Oral, topical and parenteral formulations of diclofenac is exist and is extensively used for treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and mild to moderate pain. Diclofenac has the most inhibitor effect of prostaglandin E2 production other than NSAIDs, and this inhibitor effect of diclofenac may be over 3 to 1000 times when compared to other NSAIDs [1,5].

NSAIDs are the most widely prescribed drugs in the world, they are usually used in sports medicine, as well [6,7]. Cardiac safety of the NSAIDs is still highly debated [4,6]. Although selective COX-2 inhibitor NSAIDs has low gastrointestinal side effects, it has been clearly observed that increased usage of COX-2 is associated with myocardial infarction and stroke [1]. NSAIDs caused imbalance between COX-1 and COX-2 is the reason of cardiac side effects of these drugs [3]. It is well known that diclofenac may show cardiotoxicty in human and animals [3,4]. On the other hand, cardiac response may be affected negatively by high intensity physical exercise and acute exercise may raise heart failure markers [8-10].

Troponins are considered to be specific markers of heart damage. It is considered that the levels of troponin are associated with heart cell necrosis. Troponins are part of the myocardial sarcomeric complex in the heart, and their primary task is to maintain the relationship between thick myosin and thin actin filaments. There are three different types of cardiac troponin; Troponin C, troponin T and troponin I. Troponin I inhibits actinomyosin bridge formation. The levels of cTnI increase in the myocardial injury specifically at all ages. Within 4 h after myocardial infarction an elevation is observed and remains high for about 7 d [11]. Creatine Kinase-MB (CK-MB) enzyme plays a role in cellular energy transport [12]. Creatine Kinase-MB (CK-MB) is a sub-isoenzyme of CK enzyme and defines myocardial necrosis [13]. It is considered to be one of the specific markers for determining heart damage [3]. Lactate Dehydrogenase (LDH) and Aspartate

Aminotransferase (AST) levels are also used as secondary markers in determining heart damage. However, LDH is considered to be a secondary marker with the short half-life and the fact that AST is also present in the liver, just as it is in the heart [14]. Secondary heart damage markers should be evaluated with primary damage markers [3]. Serum Aspartate Aminotransferase (AST), Alkaline Aminotransferase (ALT), Gamma Glutamyltransferase (GGT) and Alkaline Phosphatase (ALP) levels are measured to determine liver damage while Blood Urea Nitrogen (BUN) and creatinine levels are used to determine renal function [14-16].

It was hypothesized that the risk of developing cardiotoxicity could be higher when exercising together with diclofenac intake when possible cardiotoxic effects of diclofenac and the heavy exercise on heart were taken into account together [3,4,8].

The primary purpose of the current study was to determine the effect of diclofenac on cardiac damage markers in exercised and non-exercised rats. Secondly, it was to determine its effect on liver and kidney damage markers.

Materials and Methods

In the study, 28 rats (male, 120-130 g, 30 d age) were used. The research was carried out at Selcuk University Experimental Medicine Research and Application Center and the research protocol was approved by SUDAM (No: 2016-36).Animals were presented in plastic rat cages, at room temperature 23 ± 2°C, 50 ± 10% humidity, 12/12 night/day light period, daily fresh water and feed ad libitum during the experiment.

Rats were divided into 4 equal trial groups as follows:

1. **Control group**: Standard rat diet and drinking water were given as ad libitum for one week.
2. **Exercise group:** Standard rat diet and drinking water were given as ad libitum. The rats were floated for 30 min for one week.
3. **Diclofenac group:** Standard rat diet and drinking water were given as ad libitum. This group was received intraperitoneally at a dose of 13.5 mg/kg diclofenac once daily for one week [17].
4. **Diclofenac+Exercise group:** Standard rat diet and drinking water were given as ad libitum. Diclofenac was administered daily at a dose of 13.5 mg/kg intraperitoneally [17]. It was included in the 30 minute exercise program 1 h after the application of diclofenac for one week.

Exercise program was briefly summarized. The rats in the swimming exercise group were given a 30 min swimming exercise per day for 1 week in a swimming tank. The water tank temperature was filled with 25°C water and rested for 1 h and then the average water temperature was 22-25°C. At the beginning of the exercise, the rats were kept free in the water for 10 min for water adaptation and then the swimming exercise program was applied. At the end of the exercise period, the water was removed from the tank and dried with a towel.

All rats in all groups were euthanized by cervical dislocation after blood was taken from their hearts under anesthesia (ketamine 75 mg/kg, IM+xylazine 5 mg/kg, IM) at the end of one week's trial. Subsequently, serum was removed from the blood samples and CK-MB, LDH, AST, ALP, GGT, BUN and creatinine levels were measured with auto-analyser (Abbott Architecet CI8200) and rat specific cTnI level was determined with ELISA (MWGT Lambda Scan 200,Bio-Tec Instruments, Winooski, VT, ABD) reader.

The results were presented as mean ± SE. Data were evaluated with ANOVA and posthoc Duncan test (SPSS 22.0 for Windows/SPSS® Inc., Chicago, USA). $P<0.05$ level was considered as statistically significant.

Results

Effect of diclofenac on the organ damage markers of exercised and non-exercised are shown in Table 1. Cardiac damage markers cTnI and LDH levels of Diclofenac group were higher ($P<0.05$) than all other groups, whereas cTnI and LDH levels in Diclofenac+Exercise group were higher ($P<0.05$) than control and exercise groups. Statistically significantly ($P<0.05$) fluctuations were determined in the creatinine levels among groups.

Table 1. *Effect of diclofenac on the organ damage markers of exercised and non-exercised rats (mean ± SE).*

Parameters	Control	Exercise	Diclofenac	Diclofenac+Exercise
cTnI, µg/L	14.91 ± 3.40^{c}	40.36 ± 18.22^{c}	123.11 ± 11.03^{a}	78.98 ± 11.72^{b}
Ck-MB, U/L	348.00 ± 48.70	262.57 ± 13.83	412.85 ± 91.83	295.85 ± 46.40
LDH, U/L	220.14 ± 54.32^{b}	99.14 ± 11.66^{b}	1082.14 ± 231.51^{a}	1010.42 ± 263.51^{a}
AST, U/L	61.14 ± 6.18^{b}	71.28 ± 6.76^{b}	191.85 ± 45.08^{a}	69.28 ± 16.26^{b}
ALP, U/L	229.85 ± 28.48	186.28 ± 12.52	294.57 ± 145.78	543.42 ± 256.03
GGT, U/L	3.42 ± 0.57	4.00 ± 0.01	19.71 ± 12.69	34.57 ± 15.81

BUN, mg/dl	37.71 ± 2.90	41.00 ± 1.82	52.00 ± 12.78	72.71 ± 26.09
Creatinine, mg/dl	0.37 ± 0.03[a,b]	0.45 ± 0.00[a]	0.24 ± 0.04[c]	0.30 ± 0.02[b,c]

[a, b, c]Different letters in the same line are statistically significant (Duncan test, P<0.05). cTnI: Cardiac Troponin I; CK-MB: Creatine Kinase-MB; LDH: Lactate Dehydrogenase; AST: Aspartate Aminotransferase; GGT: Gamma Glutamyltransferase; ALP: Alkaline Phosphatase (ALP); BUN: Blood Urea Nitrogen.

Discussion

Diclofenac is frequently used in sports, especially because of its strong anti-inflammatory and analgesic properties [7]. It is known that NSAIDs are associated with heart failure [18].

In this study, exercise did no increase (P>0.05) any organ damage markers including heart (Table 1), though it has been mentioned that acute exercise may affect the cardiac markers [9]. This difference may be due to species diversity and/or exercise practice differences. Diclofenac administration increased (P<0.05) the level of cTnI, specific cardiac damage marker, and levels of LDH and AST, secondary cardiac damage markers, and exercise did no increased the cardiac damage markers of diclofenac (Table 1). NSAIDs including diclofenac are the most widely prescribed drugs in the world [6]. NSAIDs are usually used in sports medicine for treatment and prophylaxis purposes, whereas the prolonged prophylactic use of NSAIDs by athletes is unsafe and cardiotoxic side effects of NSAIDs are well known [5,7,18-21]. NSAIDs show these effects by disrupting the balance between COX1 and COX2 [3]. Diclofenac may show important side effects including cardiovascular [1,22]. It has been reported that diclofenac could also demonstrate its cardiotoxic effect by affecting mechanisms related to reactive oxygen species in the heart's cells [23]. Increases in cTnI, LDH and AST levels after administration of diclofenac have been reported [3]. In order to determine developing cardiac damage, LDH and AST levels are used as secondary markers while measuring the level of cTnI as a specific marker [11,14,24,25].

In this study, it was determined that exercise and diclofenac administration did not have any effect on the liver (AST, GGT), biliary tract (ALP) and kidney (BUN, creatinine) damage markers [26,27]. Elevation of ALP level was detected after diclofenac administration, but there were no changes in GGT, BUN and creatinine levels [3]. In rats, it was reported that diclofenac administration increased the BUN level at different doses when creatinine levels were not affected [28]. Considering that diclofenac is the first choice in renal colic patients and that the level of diclofenac-induced hepatotoxicity is very low, it can be stated that diclofenac is safe from the liver and kidney aspect and exercise does not increase the adverse effects on the liver and kidney [5,29].

In conclusion, it is stated that diclofenac may cause cardiac damage when used one week and its usage with exercise may not have significant adverse effects on the heart and may not cause serious adverse effects on the liver and kidney. However, when diclofenac is used in exercised and non-exercise situations, histopathologic examinations must be performed in order to clearly demonstrate the adverse effects on heart, liver and kidney.

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References

1. Gan TJ. Diclofenac: An update on its mechanism of action and safety profile. Cur Med Res Opin 2010; 7: 1715-1731.
2. Olsen SAM, Fosbol EL, Lindhardsen J, Folke F, Charlot M, Selmer C, Lamberts M, Olesen BJ, Kober L, Hansen PR, Torp-Pedersen C, Gislason GH. Duration of treatment with non-steroidal anti-inflammatory drugs and impact on risk of death and recurrent myocardial infarction in patients with prior myocardial infarction: a nationwide cohort study. Circulation 2011; 123: 2226-2235.
3. Er A, Dik B, Corum O, Cetin G. Cardiac safety of diclofenac at a single dose in ram. Sci World J 2013; 2013: 1-4.
4. Ray WA, Varas-Lorenzo C, Chung CP, Castellsague J, Murray KT, Stein M, Daugherty JR, Arbogast PG, Garcia-Rodriguez LA. Cardiovascular risks of non-steroidal anti-inflammatory drugs in patients after hospitalization for serious coronary heart disease. Circ Cardiovasc Qual Outcomes 2016; 2: 155-163.
5. Boelsterli UA. Diclofenac-induced liver injury: A paradigm of idiosyncratic drug toxicity. Toxicol Appl Pharmacol 2003; 192: 307-322.
6. Patrono C. Cardiovascular effects of non-steroidal anti-inflammatory drugs. Curr Cardiol Rep 2016; 18: 2-8.
7. Paoloni JA, Milne C, Orchard J, Hamilton B. Non-steroidal anti-inflammatory drugs in sports medicine: Guidelines for practical but sensible use. Br J Sports Med 2009; 11: 863-865.
8. Cristi-Montero C, Sanchez-Collado P, Veneroso C, Cuevas MJ, Gonzalez-Gallego J. Effect of an acute exercise bout on Toll-like receptor 4 and inflammatory mechanisms in rat heart. Rev Med Chile 2012; 140: 1282-1288.
9. Algul S, Ozcelik O. Determination of the effects of acute exercise on creatine kinase, creatine kinase-myocardial isoenzyme levels in sedentary male subjects. FU Med J Health Sci 2016; 2: 51-55.
10. Gresslien T, Agewall S. Troponin and exercise. Int J Cardiol 2016; 221: 609-621.
11. Bozkaya TA. Biomarkers in cardiovascular surgery biomarkers as early predictors for organ damage after cardiac surgery. J MU Inst Health Sci 2015; 1: 65-74.

12. Oudman I, Joseph F, Clark JF, Brewster LM. The effect of the creatine analogue beta-guanidinopropionic acid on energy metabolism: a systematic Review. Plos One 2013; 1: 1-13.
13. Meric M. Non-ST segment elevation acute coronary syndromes. J Exp Clin Med 2012; 29: 133-139.
14. Kose SI, Maden M. Biomarkers and clinical usages. Dicle Univ Vet Fak Derg 2013; 1: 30-37.
15. Ozdemir S. Routine tests of the liver. Nobel Med 2009; 2: 5-9.
16. Tasgin E, Lok S, Haliloglu S, Demir N, Ergin H. Effects of single dose administered nandrolone decanoate on serum cytokine levels and some biochemical parameters in male and female rats. Biomed Res-India 2017; 8: 3727-3730.
17. El- Maddawy Z, El-Ashmawy IM. Hepato-renal and hematological effects of diclofenac sodium in rats. Global J Pharmacol 2013; 2: 123-132.
18. Cheetham TC, Graham DJ, Campen D, Hui R, Spence M, Levy G, Shoor S. Myocardial infarction and its association with the use of non-selective NSAIDS: a nested case-control and time-to-event analysis. Perm J 2008; 1: 16-22.
19. Warden SJ. Prophylactic misuse and recommended use of non-steroidal anti-inflammatory drugs by athletes. Br J Sports Med 2009; 8: 548-549.
20. Tscholl PM, Vaso M, Weber A, Dvorak J. High prevalence of medication use in professional football tournaments including the World Cups between 2002 and 2014: a narrative review with a focus on NSAIDs. Br J Sports Med 2015; 49: 580-582.
21. Tasgin E, Lok S. Effects of exhaustive exercıse investigation of rat's ibuprofen of adverse cardiac events. Sci Mov Health 2016; 2: 696-700.
22. Kontogiorgis C, Valikeserlis I, Hadjipavlou-Litina D, Nena E, Constantinidis TC. Use of non-selective non-steroidal anti-inflammatory drugs in relation to cardiovascular events. A systematic pharmaco-epidemiological review. Curr Vasc Pharmacol 2016; 6: 502-513.
23. Ghosh R, Goswami SK, Feitoza LF, Hammock B, Gomes AV. Diclofenac induces proteasome and mitochondrial dysfunction in murine cardiomyocytes and hearts. Int J Cardiol 2016; 223: 923-935.
24. Avci Kucuk M, Bakir F, Topcuoglu C, Guctekin A. Troponin T and troponin I at acute coronary syndrome. Turk Hij Den Biyol Derg 2011; 3: 127-134.
25. Turen S, Efil S. Acute coronary syndromes and nursing management. Inten Care Nurs J 2014; 2: 43-51.
26. Turgut K. Liver tests in: Veterinary clinic laboratory diagnosis. Bahcivanlar Press, Konya, Turkey 2000; 202-257.
27. Set T, Sahin EM. Kidney function tests for primary care physicians. Sted 2003; 9: 344-348.
28. Bolat D, Selcuk ML. Stereological and biochemical evaluation of diclofenac-induced acute nephrotoxicity in rats. Revue Med Vet 2013; 6: 290-294.
29. Ay MO, Avcı A, Acehan S, Gulen M, Icme F, Sebe A. Management of patients with renal colic in emergency department. Arc Med Rev J 2014; 23, 2: 345-361.

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