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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Protective Effects of Hydroxytyrosol Mediated by Pentraxin-3 and Trpm2 on Lung Damage

Pentraxin-3 ve Trpm2 Aracılı Olan Hidroksitirozolun Akciğer Hasarı Üzerindeki Koruyucu Etkileri

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ABSTRACT

Aim: The role of Pentraxin-3 (PTX3) and Transient Receptor Potential (TRP) Melastatin-Like Subfamily Member 2 (TRPM2) in its protective impacts on the lungs in MI by administering Hydroxytyrosol (HT) to rats before Myocardial Infarction (MI) were investigated in the study.

Material and Method: The rats were divided into 4 Groups (n=7) (Control, MI 6th-hour, MI 7th-day, MI+HT 7th-day), and 200mg/kg Isoproterenol (ISO) was given subcutaneously to the rats to induce MI. The rats were given fluid containing HT 4 ml/kg/day orally for 6 weeks before MI. PTX3 and TRPM2 levels in the lung tissues were evaluated immunohistochemically.

Results: PTX3 and TRPM2 expression was detected in the smooth muscle structures and interalveolar areas of vessels and bronchioles in the lung tissue. PTX3 and TRPM2 levels increased especially on the 7th day following MI, and declined significantly on the 7th day following HT administration before MI.

Conclusion: In this study, it was concluded that HT may exert its protective effect on the lungs in MI through PTX3 and TRPM2. PTX-3 and TRPM2 may be target molecules during treatment of MI with HT.

Keywords: Myocardial Infarction, lung, Hydroxytyrosol, PTX3, TRPM2

ÖZ

Amaç: Bu çalışmada Miyokard İnfarktüsü (MI) öncesi sıçanlara Hidroksitirozol (HT) uygulanarak MI'da akciğerler üzerindeki koruyucu etkisinde Pentraxin-3 (PTX3) ve Geçici Reseptör Potansiyeli (TRP) Melastatin Benzeri Alt Aile Üyesi 2'nin (TRPM2) rolü araştırılmıştır.

Gereç ve Yöntem: Sıçanlar 4 gruba (n=7) ayrıldı (Kontrol, MI 6. saat, MI 7. gün, MI+HT 7. gün) ve MI oluşturmak için sıçanlara 200 mg/kg Isoproterenol (ISO) cilt altı uygulandı. Sıçanlara MI'dan önce 6 hafta boyunca ağızdan 4 ml/kg/gün HT içeren sıvı verildi. Akciğer dokularındaki PTX3 ve TRPM2 düzeyleri immünohistokimyasal olarak değerlendirildi.

Bulgular: Akciğer dokusunda damarların ve bronşiollerin düz kas yapılarında ve interalveoler alanlarında PTX3 ve TRPM2 ekspresyonu tespit edildi. PTX3 ve TRPM2 düzeyleri özellikle MI sonrası 7. günde artarken, MI öncesi HT uygulamasını takiben 7. günde anlamlı düşüş gösterdi.

Sonuç: Bu çalışmada HT'nin MI'da akciğerler üzerindeki koruyucu etkisini PTX3 ve TRPM2 aracılığıyla gösterebileceği sonucuna varıldı. MI'nın HT ile tedavisi sırasında PTX-3 ve TRPM2 hedef moleküller olabilir.

Anahtar Kelimeler: Miyokard İnfarktüsü, akciğer, Hidroksitirozol, PTX3, TRPM2

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INTRODUCTION

Acute Lung Injury (ALI) is a serious complication of cardiac surgery and Acute Myocardial Infarction (AMI). Myocardial Ischemia-Reperfusion (IR) injury occurs in cardiac surgery and revascularization treatment of acute myocardial infarction (1). Reactive oxygen radicals, white blood cell activation, and systemic inflammatory response might cause the damage of ischemic microcirculation and distal organ in myocardial IR. The lung is the earliest and most prominent organ that has distal organ damage following reperfusion. Myocardial IR causes ALI to increase morbidity and mortality in patients undergoing cardiac surgery (1). There is no specific and effective treatment in ALI following myocardial IR. For this reason, it is of great importance to elucidate the mechanism of ALI following myocardial IR and to search for effective treatments to avoid and treat lung injury.

Hydroxytyrosol (HT) is the strongest antioxidant in olives and olive oil. HT has a wide variety of biological activities useful for human health (2). Its antioxidant characteristics were reported in various in vitro and in vivo models and in clinical studies that were conducted in healthy subjects and patients affected by NAFLD (2). HT is a potent free radical scavenger and metal chelator, working as a chain breaker after donating one hydrogen atom to peroxyl radicals and shows significant anti-inflammatory, antimicrobial, antiatherogenic, and antithrombotic activities (3). It also has useful impacts on endothelial dysfunction, lipid, and hemostatic profiles. For this reason, it might be though of a neuroprotective, cardioprotective, and chemo-preventive compound (3).

Pentraxin 3 (PTX3) is an acute-phase protein whose plasma concentration is rapidly upregulated in inflammatory conditions such as sepsis and Acute Respiratory Distress Syndrome , with low or even undetectable blood levels in healthy individuals (3). High PTX3 levels in the blood are detected in various diseases. Pentraxin 3 recognizes and binds to many pathogens, activates the complement cascade, and plays roles in clearing apoptotic and necrotic cells (4). Elevated Pentraxin 3 levels are associated with disease severity and mortality in patients who have acute lung injury and sepsis (5). Increasing evidence suggests that PTX3 is a novel biomarker of clinical inflammation .

Many studies suggest that TRP channels may become targets for drug use for the treatment of respiratory diseases. TRPM2 channels, one of them, are found widely in bone marrow, heart, liver, pancreas, leukocytes, lungs, spleen, eye, and brain. TRPM2 is a non-selective cation channel co-activated by ADP-ribose (ADPR) and Ca2+ (6). The Ca2+ flow induced by TRPM2 controls the oxidant-induced signaling cascade that is responsible for chemokine production. This increases endothelial adhesion of neutrophils and generation of Reactive Oxygen Radicals (ROS) and exacerbates endothelial inflammation and damage (7). Continued ROS production by neutrophils causes extensive tissue damage in chronic inflammation. For this reason, TRPM2 has become an attractive pharmacological target.

The mechanisms of the protective impacts of HT on the lung, which is one of the most affected organs in MI, are not known fully. For this reason, the purpose was to investigate whether PTX3 and TRPM2 play roles in the protective impacts of HT on the lungs in MI in the present study.

MATERIAL AND METHOD

Animals and experimental design

The study was conducted by obtaining tissue ethics from Adıyaman University Experimental Animals Ethics Committee with Date: 06.10.2022, Decision no: 06. A total of 28 Sprague-Dawley (8-10-week-old) male rats, weighing 200-250 g, obtained from Adıyaman University Experimental Research Center, were used in the study. The animals were given standard water and feed in the same environment and fed ad libitum. Experimental animals were divided into 4 Groups (n=7); Group I (Control), Group II (MI 6th-hour), Group III (MI 7th-day), and Group IV (MI+HT 7th-day) (8). No application was made to the control Group in the experiment. HT was supplied in liquid form from Kale Natural Herbal Products Company (Turkey) and 4 ml/kg/day of this liquid that contained HT was administered orally for 6 weeks to the rats in Groups IV and 200 mg/kg ISO (Isoproterenol Hydrochloride, I5627, Sigma-Aldrich Corporation St. Louis, USA) was administered subcutaneously to induce MI in the rats. The rats were anesthetized with IP Ketamine (75 mg/kg) + Xylazine (10mg/kg) and blood samples were taken from the hearts in Group II at the 6th hour and on the 7th day in Groups III and IV and the experiment was terminated. The lung tissues were fixed in a 10% Formaldehyde solution for histopathological examination and the serum samples were kept at -80°C for biochemical studies.

Immunohistochemical Examination

The lung tissues of the animals were passed through routine histological follow-up series and embedded in paraffin blocks. Immunohistochemical procedures were applied as previously described by Kocaman and Artas [9]. Immunohistochemistry (IHC) was performed by using 3µm thick histological tissue microarray slides. The following antibodies were used; PTX3 Antibody (PA5-36156, Thermo Fisher Scientific, İnvitrogen, Waltham, Massachusetts, ABD) and Rabbit Polyclonal Anti-TRPM2 Antibodies (Ab-11168), Abcem, Cambridge, UK). The slides were evaluated and photographed by using the Zeiss Axio Scope A1 microscope (Carl Zeiss

Microscopy GmB H 07745 Jena, Germany). As a result of the immunohistochemical staining, histoscores were established for PTX3 and TRPM2.

The microscopic evaluation of the staining intensity was made as follows; 0 was given for negatively stained areas, 0.1 value for <25% stained areas, 0.4 value for 26-50% stained areas, 0.6 value for 51-75% stained areas, and nearly homogeneous areas (76-100%) were given a value of 0.9. The final histoscore was calculated by using the following formula; Histoscore = Distribution X Density [9].

Statistical Analysis

The SPSS version 22 (IBM Corporation, USA) was used for the analyses. The conformity of the quantitative data to the normal distribution was evaluated with the Shapiro-Wilk Test. The One-Way ANOVA Test, Post-Hoc Multiple Comparisons, and the Tukey HSD Test were used. Since the distribution was normal, ANOVA was used for comparisons between groups. The data are given as Mean ± SD and p<0.05 was considered statistically significant.

RESULTS

Immunohistochemical findings

The following findings were obtained in immunohistochemical staining for PTX-3 and TRPM2 immunoreactivity in the smooth muscle structures and interalveolar areas of the vessels and bronchioles in the lung tissue under a light microscope.

A significant increase in MI was detected on the 7th day when PTX-3 immunoreactivity in the bronchioles, alveoli and vessels in the lung tissue was compared with the control group (p<0.001). When compared with the MI day 7 Group, PTX-3 immunoreactivity declined at a statistically significant level in the MI+HT day 7 Group (p<0.001) (**Table 1**) (**Figure 1**).

Table 1. Immunohistochemical findings for PTX-3 in the lung tissues

Groups	Control	MI 6th-hour	MI 7th-day	MI+HT 7th-day
PTX-3	0.09±0.02	0.2±0.08	0.66±0.17[ab]	0.14±0.05 [c]

Error bars show SD; a. p<0.05 compared to control; b. p<0.05 compared to 6th-hour MI; p<0.05 compared to 7th-day of MI.

In terms of TRPM2 immunoreactivity, a significant increase was detected in MI on the 7th day in the alveoli in lung tissues compared to the Control Group (p<0.001). When compared with the MI day 7 Group, TRPM2 immunoreactivity declined at a statistically significant level in the MI+HT day 7 Group (p<0.001) (**Table 2**) (**Figure 2**).

Table 2. Immunohistochemical findings for TRPM2 in the lung tissues

Groups	Control	MI 6th-hour	MI 7th-day	MI+HT 7 th-day
TRPM2	0.06±0.02	0.06±0.03	0.77±0.16[ab]	0.09±0.02[c]

Error bars show SD; a. p<0.05 compared to control; b. p<0.05 compared to 6th-hour MI; p<0.05 compared to 7th-day of MI.

DISCUSSION

The role of PTX3 and TRPM2 in the effect of HT, which benefits from its protective effect on the cardiovascular system, on the lungs, which is one of the most affected organs in MI, was investigated histopathologically in the lungs of the rats with MI induced with ISO in the study and it was shown for the first time that PTX3 and TRPM2 may be involved in this effect mechanism histopathologically in the lungs with the application of HT before MI.

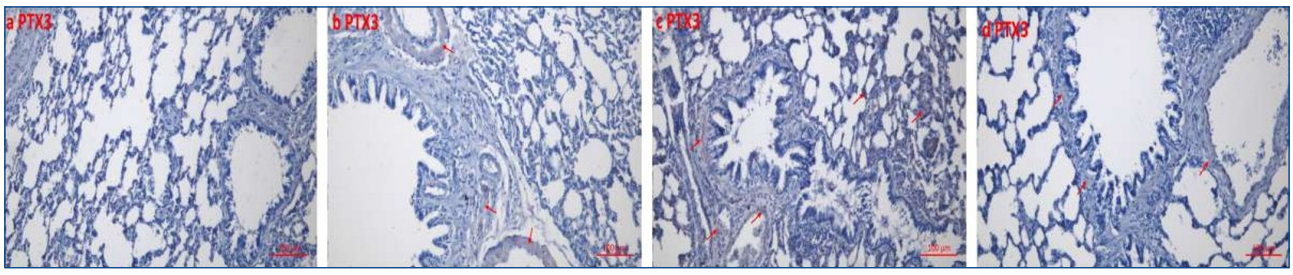

Figure 1. Immunohistochemical findings for PTX-3 in the lung tissue (a.Control, b.MI 6th-hour, c.MI 7th-day,d.MI+HT 7th-day)

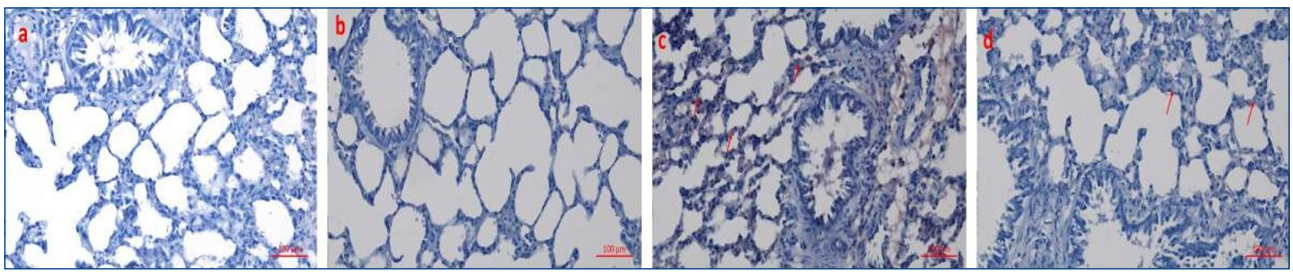

Figure 2. Immunohistochemical findings for TRPM2 in the lung tissue (a.Control, b.MI 6th-hour, c.MI 7th-day, d.MI+HT 7th-day)

PTX3 is a novel member of the long Pentraxins. AMI is an acute phase protein, which is also a novel serological marker that reflects tissue inflammation and damage in various human pathologies (10). In a human study, elevated PTX3 expression was shown in bronchial biopsies of allergic asthmatic individuals compared to healthy controls (11). Also, serum levels of PTX3 are higher in COPD patients compared to people who have lung cancer (12). Abnormal elevations of circulating PTX3 are associated with the severity of acute lung injury and lung allograft dysfunction (13). Genetic variations in PTX3 appear to be predictors of pulmonary microbial infections (14). However, strong and overlapping associations were demonstrated between the severity of airflow obstruction, emphysema and mortality, and lung and blood PTX3 levels and PTX3 regulatory gene variants in smokers (15). PTX 3 has moderate accuracy in diagnosing respiratory tract infections and ventilator-associated pneumonia (16). However, PTX 3 knockout mice were shown to suffer more severe endotoxin-induced lung injury when compared to wild-type controls. It was reported that Pentraxin 3 overexpression was detrimental to the survival of mice suffering from ischemia and reperfusion injury (4).

TRPM2 is a non-selective cation channel that is activated mostly by heat, ROS, intracellular Ca2+, and ADP-Ribose (ADPR) in inflammatory processes. TRPM2-mediated Ca2+ signaling is important for macrophage activation and phagocytic functions. It was reported that disabling TRPM2 reduced ROS production in macrophages along with tissue damage in a mouse model of lung injury (17). In another study, it was reported that TRPM2 functions as an oxidant sensor in the cardiovascular and pulmonary artery endothelium and may play key roles in leukocyte activation, vascular endothelial permeability, and injury (18). TRPM2 was reported to be responsible for mediating the impacts of oxidants on the endothelium by mechanisms involving the production of second messenger ADP ribose in human pulmonary artery endothelial cells (18). Oxidative stress is initiated by the products of active lung macrophages and infiltrating neutrophils (19). In particular, oxidant stress through the production of oxygen metabolites such as H2O2 and chemotactic cytokines increases endothelial adhesion of neutrophils and vascular endothelial permeability as critical factors that govern tissue edema formation and neutrophil extravasation (20). Chemokine expression is inducible and is responsible for the recruitment of inflammatory cells to sites of infection or injury (21). A recent study has reported the functional role of monocyte TRPM2 channels in mediating chemokine production and neutrophil-induced lung injury (7).

It was determined in the present study that PTX3 and TRPM2 levels elevated in the lung tissue, especially on the 7th day following MI, and declined on the 7th day following HT administration before MI, which suggests that HT may exert its protective impacts on the lungs in MI over PTX3 and TRPM2, which are molecules associated with inflammation. It was reported in many studies that HT protects against cardiovascular diseases because of a wide variety of biological activities such as endothelial dysfunction and prevention of macrophage activation, limits Low-Density Lipoprotein (LDL) oxidation, regulates blood lipid profile, reduces platelet aggregation and chronic inflammation (22). In vivo evidence is associated with the activation of the SIRT/MAPK Signaling Pathway, which supports that HT has protective impacts on lung inflammation over autophagy, which is a type of lung inflammation (23). It was shown that HT treatment provides antioxidant, anti-inflammatory, and anti-proliferative impacts by protecting against irradiation-induced pulmonary fibrosis in rats (24). Based on this, it is considered that PTX3 and TRPM2 are likely to be involved in the anti-inflammatory and antioxidant action mechanisms of HT. However, this needs to be supported by more molecular studies.

The limitation of the study was that the results were not supported by biochemical and genetic examinations. Future studies describing the relationship of HT with PTX3 and TRPM2 will provide a better understanding of the molecular mechanisms of the present study. Supporting these findings with clinical studies is also important in terms of demonstrating the protective impacts of HT on humans.

CONCLUSION

In conclusion, in the present study, it was considered that HT may exert its protective impacts on the lungs in MI by reducing PTX3 and TRPM2. The findings may hold promise for the treatment of the lungs, which is one of the organs where distant tissue damage is most common following MI.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted by obtaining tissue ethics from Adıyaman University Experimental Animals Ethics Committee with Date: 06.10.2022, Decision no: 06.

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. Zhang W, Guo Y, Yu S, Wei J, Jin J. Effects of edaravone on the expression of β-defensin-2 mRNA in lung tissue of rats with myocardial ischemia reperfusion. Mol Med Rep 2013;7(5):1683-87.
2. Martínez-Zamora L, Peñalver R, Ros G, Nieto G. Olive Tree Derivatives and Hydroxytyrosol: Their Potential Effects on Human Health and Its Use as Functional Ingredient in Meat. Foods 2021;10:2611.
3. Smeriglio A, Denaro M, Mastracci L et al. Safety and efficacy of hydroxytyrosol-based formulation on skin inflammation: In vitro evaluation on reconstructed human epidermis model. Daru 2019;27:283–93.
4. Townsend M, Fowler B, Aulakh GK, Sing B. Expression of pentraxin 3 in equine lungs and neutrophil. The Canadian Journal of Veterinary Research 2023;87:9–16.
5. Bilgin H, Haliloglu M, Yaman A et al. Sequential measurements of pentraxin 3 serum levels in patients with ventilator-associated pneumonia: a nested case-control study. Can J Infect Dis Med Microbiol 2018;2018:4074169.
6. Szollosi A. Two Decades of Evolution of Our Understanding of the Transient Receptor Potential Melastatin 2 (TRPM2) Cation Channel. Life (Basel) 2021; 11(5):397.
7. Yamamoto S, Shimizu S, Kiyonaka S et al. TRPM2-mediated Ca2+ influx induces chemokine production in monocytes that aggravates inflammatory neutrophil infiltration. Nature Med 2008; 14:738–47
8. Erten M, Çimenci İG, Kuloğlu T, Kalaycı M, Erten F. The relationship between visfatin and cardiac markers on induced myocardial infarction in rats. Cytokine 2019;115: 116-20.
9. Kocaman N, Artas G. Can novel adipokines, asprosin and meteorin-like, be biomarkers for malignant mesothelioma? Biotech Histochem 2020;95(3):171-75.
10. Elmahalawy II, Ammar AS, Fathy WM, Salama AE, Mokhtar WS. Pentraxin 3 as an early marker in diagnosis of ventilator associated pneumonia. Egypt J Chest Dis Tuberc 2017;66:709–12.
11. 11. Zhang J, Shan L, Koussih L et al. Pentraxin 3 (PTX3) Expression in Allergic Asthmatic Airways: Role in Airway Smooth Muscle Migration and Chemokine Production. PLoS One 2012;7(4): e34965.
12. Jie H, Li Y, Pu X, Ye J. Pentraxin 3, a predicator for 28-day mortality in patients with septic shock. Am J Med Sci 2017;353:242–6.
13. Bilgin H, Haliloglu M, Yaman A et al. Sequential measurements of pentraxin 3 serum levels in patients with ventilator-associated pneumonia: a nested case-control study. Can J Infect Dis Med Microbiol 2018;2018:4074169.
14. Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J.The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. J BMC Med Res Methodol 2003;3:25.
15. Zhang Y, Tedrow J, Nouraie M et al. Elevated plasma level of Pentraxin 3 is associated with emphysema and mortality in smokers. Thorax 2021;76(4): 335–42.
16. Ye W, Huang QD, Tang TY, Qin GY. Diagnostic value of pentraxin 3 in respiratory tract infections. Medicine 2020;99:14.
17. Zong P,Feng J, Yue Z et al. TRPM2 deficiency in mice protects against atherosclerosis by inhibiting TRPM2-CD36 inflammatory axis in macrophages. Nat Cardiovasc Res 2022;1:344–60.
18. Hecquet CM, Ahmmed GU, Vogel SM, Malik AB. Role of TRPM2 Channel in Mediating H2O2-Induced Ca2+ Entry and Endothelial Hyperpermeability. Circ Res 2008;102:347–55.
19. Johnson A, Phillips P, Hocking D, Tsan MF, Ferro T. Protein kinase C inhibitor avoids pulmonary edema in response to H2O2. Am J Physiol Heart Circ Physiol 1989; 256:1012–22.
20. Lum H, Roebuck K. Oxidant stress and endothelial cell dysfunction. Am J Physiol Cell Physiol 2001;280:719–41.
21. Han XB, Liu X, Hsueh W, Plaen IG. Macrophage inflammatory protein-2 mediates the bowel injury induced by platelet-activating factor. Am J Physiol Gastrointest Liver Physiol 2004;287: 1220–26.
22. Cunha C, Aversa F, Lacerda JF et al. Genetic PTX3 deficiency and aspergillosis in stem-cell transplantation. N Engl J Med 2014;370:421–32.
23. Yang X, Jing T, Li Y et al. Hydroxytyrosol Attenuates LPS-Induced Acute Lung Injury in Mice by Regulating Autophagy and Sirtuin Expression. Curr Mol Med 2017;17:149–59.
24. Liu ZH, Fan W, Chen RC. 3,4-dihydroxyphenylethanol suppresses irradiation-induced pulmonary fibrosis in adult rats. Int J Clin Exp Pathol 2015;8(4):3441–50.