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Protective Effects of the Purinergic Receptor Antagonist A438079 on Lipopolysaccharide-Induced Kidney Injury in Rats

Pürinerjik Reseptör Antagonisti A438079'un Sıçanlarda LPS'nin Neden Olduğu Böbrek Hasarı Üzerindeki Koruyucu Etkileri

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ABSTRACT

Sepsis is an important clinical problem associated with organ dysfunction and high mortality. To investigate the effects of this purinergic receptor on renal dysfunction in sepsis, a rat model was created by the intraperitoneal injection of lipopolysaccharides. The groups were control, A438079, lipopolysaccharides, and lipopolysaccharide+A438079. Following lipopolysaccharide (8 mg/kg) injection, A438079 (15 mg/kg) was administrated (intraperitoneally) in the study groups. Then, blood and kidney tissues were collected for analysis from rats sacrificed under anesthesia. In the histopathological evaluations, it was observed that A438079 reduced the damage caused by sepsis. It was observed that the creatinine and blood urea nitrogen) concentrations of the serum samples belonging to the lipopolysaccharide+A438079 group were lower than the serum samples belonging to the lipopolysaccharide group. Aquaporin-2, Bcl-2, caspase-3, and NfkB-p65 protein expressions in kidney tissues were evaluated by western blot. On the other hand, it was observed that there was a decrease in the expression of these proteins in the A438079-treated groups. In conclusion, A438079 can reduce the inflammatory response in acute kidney injury induced by lipopolysaccharides and improve renal dysfunction.

Keywords: A438079, LPS, purinergic receptor, rats, sepsis

ÖZ

Sepsis, organ disfonksiyonu ve yüksek mortalite ile ilişkili önemli bir klinik problemdir. Bu pürinerjik reseptörün sepsiste böbrek fonksiyon bozukluğu üzerindeki etkisini araştırmak için intraperitoneal LPS (Lipopolisakarit) enjeksiyonu ile sıçan modeli oluşturuldu. Çalışma Kontrol, A438079, LPS, LPS ve LPS+A438079 gruplarından oluşmaktaydı. Çalışma gruplarına LPS (8 mg/kg) enjeksiyonunu takiben A438079 (15 mg/kg) uygulandı (i.p). Daha sonra anestezi altında sakrifiye edilen ratlardan analizler için kan ve böbrek dokuları toplandı. Histopatolojik değerlendirmelerde A438079'un sepsisin neden olduğu hasarı azalttığı görüldü. LPS+A438079 grubuna ait serum örneklerinin kreatinin ve BUN (Blood Urea Nitrogen) konsantrasyonlarının LPS grubuna ait serum örneklerine göre daha düşük olduğu belirlendi. Böbrek dokularında AQP2, Bcl-2, Kaspaz-3 ve NfkB-p65 protein ekspresyonları western blot ile değerlendirildi. Öte yandan, A438079 ile tedavi edilen gruplarda bu proteinlerin ifadesinde azalma olduğu gözlendi. Sonuç olarak, A438079, LPS'nin neden olduğu akut böbrek hasarında enflamatuar yanıtı azaltabilir ve böbrek fonksiyon bozukluğunu iyileştirebileceği belirlenmiştir.

Anahtar Kelimeler: A438079, LPS, pürinerjik reseptör, sıçanlar, sepsis

Introduction

Sepsis has many causes, including serious infections, kidney disease, and some types of cancer. The mortality rates of sepsis are still more significant, and septic shock continues to be the major cause of

death despite the development of numerous therapeutic medicines for clinical use (Morrell et al., 2009). When bacteria, viruses, or parasites invade the bloodstream, the immune response is initiated, and the immune system is triggered (Pinsky, 1996). Excessive triggering of defense mechanisms leads to severe inflammation or septic shock (Mccabe et al., 1983; Morrison & Ulevitch, 1978). It was reported in 1988 that endotoxic shock is not triggered by toxins secreted from microorganisms. But an endotoxic shock results in a strong, uncontrollable immune response in the host (Ceramil & Beutler, 1988). Incorporation of lipopolysaccharides (LPSs), essential components of Gram-negative bacteria, into the bloodstream induces cytokines [such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β)] and excessive endogenous proinflammatory mediators. Leukotrienes and thromboxane A2 are involved in sepsis/septic shock-related responses (Beasley et al., 1989).

Activation of Toll-like receptors in monocyte, macrophage, and microglial cells by LPSs induces a large amount of an inflammatory component, pro-IL-1β, in the cytoplasm. Activation of P2X7 receptors by adenosine triphosphate (ATP) triggers potassium influx, procaspase-1 cleavage, conversion of pro-IL-1β to mature IL-1β, and release of mature IL-1β into the extracellular environment (Ferrari et al., 2007; Perregaux & Gabel, 1994; Sanz & Virgilio, 2000). In vivo and in vitro experiments in P2X7 receptor-knockout mice have definitively identified the receptor responsible for ATP-dependent IL-1 release upon LPS loading as the P2X7 receptor (Ferrari et al., 1997; Labasi et al., 2002; Solle et al., 2001).

Since these cytokines appear to be key factors in organ dysfunction, it is thought that the inhibition of these factors may reduce organ damage. In recent years, considerable effort has been devoted to the development of selective P2X7R antagonists. One of the human and rodent P2X receptor antagonists is A438079 (Jacobson & Müller, 2016). A438079 is an antagonist that selectively binds to the P2X7 receptor. It also has potency in the nanomolar concentration range and shows high selectivity for P2X7 compared to other P2X subtypes (Tancan, 2018). Half of the cases of acute kidney injury are associated with sepsis (Uchino et al., 2005). Several preclinical models of acute kidney damage have demonstrated protective benefits of pharmacological inhibition and/or genetic deletion. (Turner et al., 2014). P2X7-knockout mice modeled for sepsis by the cecal ligation and puncture model were observed to have a weaker inflammatory response and decreased lung damage compared to wild-type mice (Santana et al., 2015).

We hypothesized that there is a relationship between ATP-sensitive P2X7 receptor expression, cytokine production, and inflammatory response in LPS-induced kidney injury of rats. We aimed to evaluate the effects of P2X7R antagonism on acute kidney injury with an LPS-induced sepsis model.

Methods

Experimental Housing

In the study, 24 Sprague–Dawley rats were divided into four equal groups. The groups consisted of the control group (no application), A438079 group [15 mg/kg intraperitoneal (i.p.) administration], LPS group (8 mg/kg *Escherichia coli* LPS administered intraperitoneally), and the A438079 + LPS group (8 mg/kg LPS and 15 mg/kg dose A438079 administered intraperitoneally). The study was approved by the Atatürk University Faculty of Veterinary Medicine Ethical Committee (date: January 24, 2023, number: E-36643897-000-2300028882).

Drug Administration

Lipopolysaccharides were dissolved in saline and given to animals at a dose of 8 mg/kg body weight (i.p.) and A438079 was dissolved in dimethyl sulfoxide (DMSO) and given to animals at a dose of 15 mg/kg body weight (i.p.) (Arulkumaran et al., 2018). A438079 was given to animals before the LPS injection. Animals were sacrificed under deep anesthesia for 6 hours in accordance with the study design, and blood and kidney tissue samples were taken for the analysis of biochemical, histological, and western blot. The procedure of the experiment is presented in Table 1.

Biochemical Analyses

Serum levels of creatinine and blood urea nitrogen (BUN) were analyzed via an auto-analyzer device Siemens Atellica CH 930 Analyzer (Siemens Healthineers, Erlangen, Germany) with rat-compatible kits to determine kidney function tests from the obtained serum samples.

Western Blot Analysis

Before western blot analysis, the acquired lung tissue samples were kept at −80°C in a deep freezer. The lung tissue samples were weighted and crushed in nitrogen gas, treated with radioimmunoprecipitation buffer (EcoTech Biotechnology, Erzurum, Turkey) supplemented with protease and phosphatase inhibitors, and homogenized using a tissue lyser device (Qiagen Inc., Valencia, CA, USA) at 30 Hz for 20 seconds to determine the relative protein expressions of Bcl-2, caspase-3, and Nf-kB-p65. A protein assay kit was used to quantify the total protein of the lung tissues (Pierce BCA, Thermo Sci., Rockford, USA). About 30 µg of protein was then put into the polyvinylidene difluoride (PVDF) membrane after being separated by 10% SDS-PAGE. First, at room temperature, 5% bovine serum albumin was used to block the membranes for 90 minutes. Then, the membranes were incubated at 4°C overnight with the appropriate primary antibodies [(IL-1β (sc-52012, Santa Cruz Biotechnology, Santa Cruz, CA, USA), TNF-α (sc-52746, Santa Cruz), P2X7R antibody (11144-1-AP, 1:20, Proteintech, Chicago, IL, USA), caspase-3 (sc-56053, Santa Cruz), Bcl-2 (sc-7382, Santa Cruz), Nf-kB-p65 (sc-109, Santa Cruz), and beta-actin (sc-47778, Santa Cruz)]. After primary antibody incubation, the PVDF membranes were washed with TBST and then incubated for an additional 90 minutes at room temperature with the second antibody (Santa Cruz, sc-2004/sc-2005) coupled with horseradish peroxidase. Then, the protein bands were captured using the enhanced chemiluminescence reagent Western ECL substrate (Thermo, 3405) and visualized and analyzed by Image Lab™ Software (Bio-Rad, Hercules, Calif, USA).

Statistical Analysis

Statistical Package for Social Sciences version 25.0 (IBM SPSS Corp., Armonk, NY, USA) package program was used for statistical analyses of data. Normality of data was determined with

Table 1.
Applications and Procedures of the Study

Groups	Applications	Scarification
Control ($n = 6$)	—	End of the study
A438079 ($n = 6$)	15 mg/kg (i.p.) A438079	Beginning of the study
LPS ($n = 6$)	10 mg/kg (i.p.) LPS	After 6 hours
A438079 + LPS ($n = 6$)	15 mg/kg (i.p.) A438079 + 10 mg/kg (i.p.) LPS	After 6 hours

Note: i.p. = intraperitoneal; LPS = lipopolysaccharide.

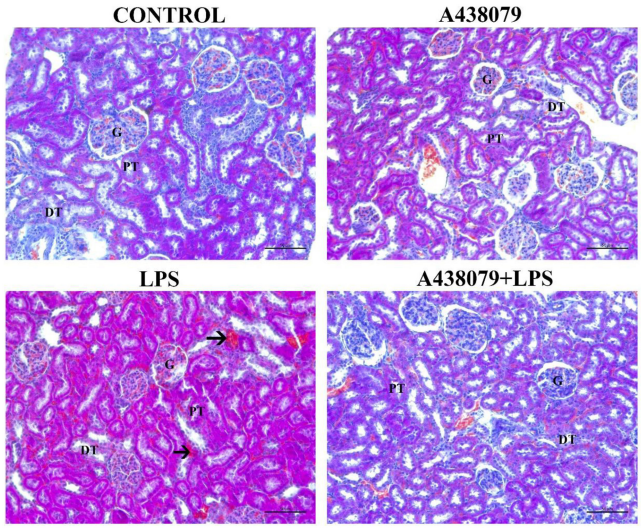

Figure 1.
Illustration of Histological Analysis of Rat Kidney Tissues for All Groups. Arrow Indicates Erythrocyte Extravasation. Crossman's Triple Stain, ×20 Magnification.

Shapiro–Wilk test, and the data are expressed as mean ± standard deviation. One-way analysis of variance test and the post-hoc Tukey test were performed to compare groups. p values ≤.05 at the 95% CI were considered statistically significant.

Results

Histopathological Results

In the kidney sections of the control group, it was observed that the cortex and medulla of the kidney tissue could be differentiated, and the fibrous capsule was intact. It was determined that the renal corpuscles in the cortex were in the normal structure, and the parietal and visceral leaves in the Bowman's capsule were in the normal histological structure. It was observed that structures such as proximal tubule and distal tubule were normal and that epithelial cells preserved their characteristic structure, features, and borders. Also, no cell infiltration and loss of brush border were observed. In the LPS group, loss of brush border in the proximal tubule cells, tubular atrophy–tubular dilatation, and increased mononuclear cell infiltration in the peritubular area compared to the control group were observed. In some areas of the cortex, vasodilation and erythrocyte extravasation were observed. The loss of brush border, tubular dilatation, tubular atrophy, and cell debris in the tubule lumen were observed to be less in the A438079 + LPS group compared to the LPS group. It was observed that erythrocyte extravasation, mononuclear lymphocyte cell infiltration, and tubular changes were decreased in the A438079 + LPS group compared to the LPS group (Figure 1).

Serum Biochemical Results

In the evaluation of serum renal function test analysis, no significant difference was determined between the control and A438079 groups, but the creatinine value in the LPS group was found to be higher than the control, A438079, and A438079 + LPS groups ($p < .05$). When the serum BUN value was examined, it was determined that it was similar in the control and A438079 groups, while it was found that this value increased significantly in the LPS and A438079 + LPS groups ($p < .05$). Also, no significant difference was found between LPS and A438079 + LPS groups ($p > .05$). Serum creatine and BUN values and comparisons of all groups are presented in Figure 2.

Western Blot Analysis Results

In the evaluation of kidney tissue protein analysis, aquaporin-2 (AQP2) and Bcl-2 levels were found to be similar in the control, A438079, and A438079 + LPS groups, while a significant decrease was determined in the LPS group compared to these groups ($p < .05$). While caspase-3 and NfkB-p65 protein levels were found to be similar in the control, A438079, and A438079 + LPS groups, a significant increase was detected in the LPS group ($p < .05$). Relative protein expression levels and comparisons of all groups are presented in Figure 3.

Discussion

The function of a selective P2X7R inhibitor (A438079) has not been thoroughly examined in an in vitro sepsis model in which treatment is administered after the LPS-induced sepsis model. To this end, we investigated the role of purinergic signaling in sepsis-associated kidney injury, considering the proinflammatory effects of the P2X7 receptor antagonist.

P2X7 receptor-related immune responses were reported in previous studies (Bours et al., 2006; Coutinho-Silva et al., 2009; da Silva et al., 2008; Di Virgilio et al., 2001; Goncalves et al., 2006; Solle et al., 2001). Despite many studies, the physiological role of this P2X7R remains unclear in the sepsis-induced kidney tissue. It has been reported to have an important role in various cellular activities, including giant cell formation (Chiozzi et al., 1997), fertilization (Foresta et al., 1996), lymphocyte proliferation (Baricordi et al., 1999), cell death (Ferrari et al., 1999; Murgia et al., 1992), and killing of invading mycobacteria (Lammas et al., 1997). The ligand-gated P2X7 receptor of ATP is thought to contribute to the development of an exacerbated inflammatory response in sepsis

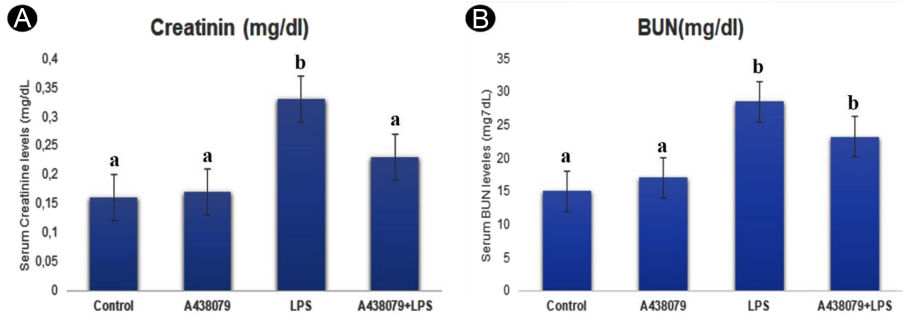

Figure 2.
Serum Kidney Function Tests for All Groups: (A) Serum Creatinine Levels and (B) Serum Blood Urea Nitrogen (BUN) Levels. The Letters a and b Indicate the Statistical Differences Between Groups.

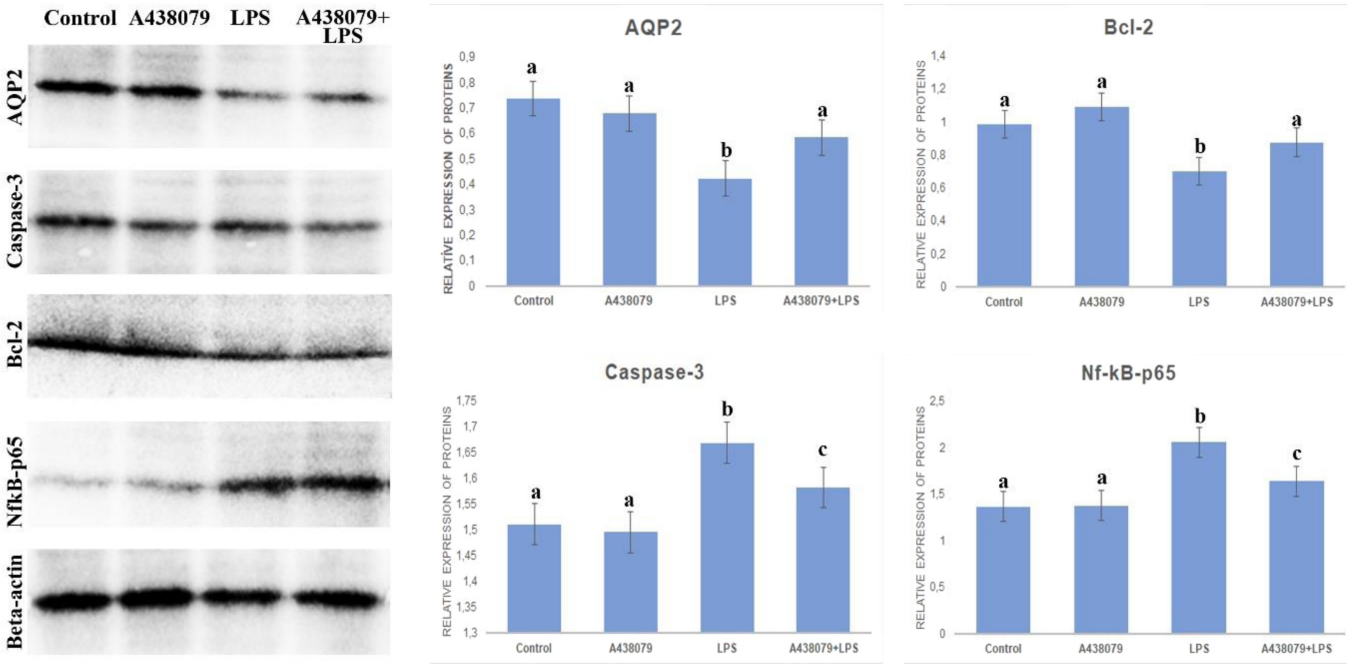

Figure 3.
Relative Protein Expression Levels of Aquaporin-2 (AQP2), Bcl-2, Caspase-3, and NfkB-p65 for All Groups. The Lowercase Letters Indicate the Statistical Differences Between Groups. p < .05 is Accepted as Significant.

(Santana et al., 2015). ATP is a chemotactic factor for neutrophils and macrophages, and the P2X7 receptor has been shown to release ATP as well as being activated by this nucleotide (Pellegatti et al., 2011; Trautmann, 2009). Neutrophils are the major inflammatory cells responsible for the immune response during sepsis (Serbina et al., 2008; Trautmann, 2009). Extracellular ATP/P2X7 receptor blockade has been shown to protect renal tubular epithelial cells from ischemia–reperfusion injury through the regulation of Nod-like receptor protein 3 (NLRP3) inflammation (Qian et al., 2021).

Studies reported that the potential benefit of P2X7 inhibition or genetic deletion in sepsis of knockout mice (Santana et al., 2015; Savio et al., 2017a,b). Another study demonstrated increased mortality with *E. coli* urosepsis in P2X7-deficient mice (Greve et al., 2017). After the onset of sepsis, inhibition of P2X7 by A438079 has been shown to reduce the early expression of renal IL-1β and abolish the increase in serum creatinine concentration at 24 hours (Arulkumaran et al., 2018). In LPS-induced sepsis of Madin–Darby canine kidney, renal tubular epithelial cells express Toll-like receptor 4, NLRP3, caspase-1, and IL-1β mRNA (Jalilian et al., 2012).

Ischemia–reperfusion model was established in mouse kidneys, and administration of A438079 immediately or 6 hours after the start of reperfusion was determined to significantly reduce serum BUN and creatinine concentrations. But, A438079 administration 24 hours after reperfusion did not affect serum BUN and creatinine concentrations (Yan et al., 2015). In accordance with the literature, this study shows that A438079 can reduce renal dysfunction in sepsis by inhibiting P2X7R.

In the study, the AQP2 expression decreased significantly in LPS-induced kidney damage, but there was an increase in the AQP2 expression in the kidney tissue of the A438070 + LPS group. In an in vivo and in vitro study, A438079 was shown to inhibit P2X7R, promoting apoptosis via Bcl-2/caspase-9/caspase-3 and inhibiting pyroptosis via NLRP3/caspase (Zhang et al., 2021). According to the results of the study, it was seen that increased expression A438079 of Bcl-2 was compatible with the literature. A decrease in the expression of reactive oxygen species (ROS), Bax, and caspase-3 was determined by the anti-apoptotic effect of A438079 (Zhu et al., 2019). In the cisplatin-induced nephrotoxicity study, significant reductions in the protein levels of p53 and caspase-3 were observed after A438079 treatment (Zhang et al., 2014). In the current study, it was observed that A438079 treatment considerably reduced the caspase-3 protein level, which was supported by the results of other studies.

Conclusion and Recommendations

In conclusion, sepsis is a complication with a high mortality rate today. Despite intense efforts and many clinical trials, little has changed in the optimal treatment since the 1960s. A438079 is an inhibitor of the purinergic receptor and alleviates the LPS-induced kidney injuries in rats by inhibiting the P2X/R-mediated inframammary response. The remarkable results in this in vivo sepsis model indicate the therapeutic efficiency of A438079; further studies targeting P2X7 in sepsis are needed.

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References

Arulkumaran, N., Sixma, M. L., Pollen, S., Ceravola, E., Jentho, E., Prendecki, M., Bass, P. S., Tam, F. W. K., Unwin, R. J., & Singer, M. (2018). P2X7 receptor antagonism ameliorates renal dysfunction in a rat model of sepsis. *Physiological Reports*, *6*(5), e13622. [CrossRef]

Baricordi, O. R., Melchiorri, L., Adinolfi, E., Falzoni, S., Chiozzi, P., Buell, G., & Di Virgilio, F. (1999). Increased proliferation rate of lymphoid cells transfected with the P2X7 ATP receptor. *Journal of Biological Chemistry*, *274*(47), 33206–33208. [CrossRef]

Beasley, D., Cohen, R. A., & Levinsky, N. G. (1989). Interleukin 1 inhibits contraction of vascular smooth muscle. *Journal of Clinical Investigation*, *83*(1), 331–335. [CrossRef]

Bours, M. J., Swennen, E. L., Di Virgilio, F., Cronstein, B. N., & Dagnelie, P. C. (2006). Adenosine 5′-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacology and Therapeutics*, *112*(2), 358–404. [CrossRef]

Cerami, A., & Beutler, B. (1988). The role of cachectin/TNF in endotoxic shock and cachexia. *Immunology Today*, *9*(1), 28–31. [CrossRef]

Chiozzi, P., Sanz, J. M., Ferrari, D., Falzoni, S., Aleotti, A., Buell, G. N., Collo, G., & Di Virgilio, F. D. (1997). Spontaneous cell fusion in macrophage cultures expressing high levels of the P2Z/P2X7 receptor. *Journal of Cell Biology*, *138*(3), 697–706. [CrossRef]

Coutinho-Silva, R., Corrêa, G., Sater, A. A., & Ojcius, D. M. (2009). The P2X 7 receptor and intracellular pathogens: A continuing struggle. *Purinergic Signalling*, *5*(2), 197–204. [CrossRef]

Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J. M., Morelli, A., Torboli, M., Bolognesi, G., & Baricordi, O. R. (2001). Nucleotide receptors: An emerging family of regulatory molecules in blood cells. *Blood*, *97*(3), 587–600. [CrossRef]

Ferrari, D., Chiozzi, P., Falzoni, S., Dal Susino, M., Melchiorri, L., Baricordi, O. R., & Di Virgilio, F. (1997). Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. *Journal of Immunology*, *159*(3), 1451–1458. [CrossRef]

Ferrari, D., Los, M., Bauer, M. K., Vandenabeele, P., Wesselborg, S., & Schulze-Osthoff, K. (1999). P2Z purinoreceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death. *FEBS Letters*, *447*(1), 71–75. [CrossRef]

Ferrari, D., Pizzirani, C., Adinolfi, E., Lemoli, R., Curti, A., Idzko, M., Panther, E., & Di Virgilio, F. (2007). The P2X7 receptor: A key player in IL-1 processing and release. *Journal of Immunology*, *179*(12), 8569–8569.

Foresta, C., Rossato, M., Chiozzi, P., & Di Virgilio, F. (1996). Mechanism of human sperm activation by extracellular ATP. *American Journal of Physiology*, *270*(6), C1709–C1714. [CrossRef]

Gonçalves, R. G., Gabrich, L., Rosário Jr., A., Takiya, C. M., Ferreira, M. L., Chiarini, L. B., Persechini, P. M., Coutinho-Silva, R., & Leite Jr., M. (2006). The role of purinergic P2X7 receptors in the inflammation and fibrosis of unilateral ureteral obstruction in mice. *Kidney International*, *70*(9), 1599–1606. [CrossRef]

Greve, A. S., Skals, M., Fagerberg, S. K., Tonnus, W., Ellermann-Eriksen, S., Evans, R. J., Linkermann, A., & Praetorius, H. A. (2017). P2X1, P2X4, and P2X7 receptor knock out mice expose differential outcome of sepsis induced by α-haemolysin producing Escherichia coli. *Frontiers in Cellular and Infection Microbiology*, *7*, 113. [CrossRef]

Jacobson, K. A., & Müller, C. E. (2016). Medicinal chemistry of adenosine, P2Y and P2X receptors. *Neuropharmacology*, *104*, 31–49. [CrossRef]

Jalilian, I., Spildrejorde, M., Seavers, A., Curtis, B. L., McArthur, J. D., & Sluyter, R. (2012). Functional expression of the damage-associated molecular pattern receptor P2X7 on canine kidney epithelial cells. *Veterinary Immunology and Immunopathology*, *150*(3–4), 228–233. [CrossRef]

Labasi, J. M., Petrushova, N., Donovan, C., McCurdy, S., Lira, P., Payette, M. M., Brissette, W., Wicks, J. R., Audoly, L., & Gabel, C. A. (2002). Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. *Journal of Immunology*, *168*(12), 6436–6445. [CrossRef]

Lammas, D. A., Stober, C., Harvey, C. J., Kendrick, N., Panchalingam, S., & Kumararatne, D. S. (1997). ATP-induced killing of mycobacteria by human macrophages is mediated by purinergic P2Z (P2X7) receptors. *Immunity*, *7*(3), 433–444. [CrossRef]

Marques da Silva, C., Miranda Rodrigues, L., Passos da Silva Gomes, A., Mantuano Barradas, M., Sarmento Vieira, F., Persechini, P. M., & Coutinho-Silva, R. (2008). Modulation of P2X7 receptor expression in macrophages from mineral oil-injected mice. *Immunobiology*, *213*(6), 481–492. [CrossRef]

Mccabe, W. R., Treadwell, T. L., & De Maria Jr., A. (1983). Pathophysiology of bacteremia. *American Journal of Medicine*, *75*(1B), 7–18. [CrossRef]

Morrell, M. R., Micek, S. T., & Kollef, M. H. (2009). The management of severe sepsis and septic shock. *Infectious Disease Clinics of North America*, *23*(3), 485–501. [CrossRef]

Morrison, D. C., & Ulevitch, R. J. (1978). The effects of bacterial endotoxins on host mediation systems. A review. *American Journal of Pathology*, *93*(2), 526–618.

Murgia, M., Pizzo, P., Steinberg, T. H., & Di Virgilio, F. (1992). Characterization of the cytotoxic effect of extracellular ATP in J774 mouse macrophages. *Biochemical Journal*, *288*(3), 897–901. [CrossRef]

Pellegatti, P., Falzoni, S., Donvito, G., Lemaire, I., & Di Virgilio, F. (2011). P2X7 receptor drives osteoclast fusion by increasing the extracellular adenosine concentration. *FASEB Journal*, *25*(4), 1264–1274. [CrossRef]

Perregaux, D., & Gabel, C. A. (1994). Interleukin-1 beta maturation and release in response to ATP and Nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. *Journal of Biological Chemistry*, *269*(21), 15195–15203. [CrossRef]

Pinsky, M. R. (1996). Organ-specific therapy in critical illness: Interfacing molecular mechanisms with physiological interventions. *Journal of Critical Care*, *11*(2), 95–107. [CrossRef]

Qian, Y., Qian, C., Xie, K., Fan, Q., Yan, Y., Lu, R., Wang, L., Zhang, M., Wang, Q., Mou, S., Dai, H., Ni, Z., Pang, H., & Gu, L. (2021). P2X7 receptor signaling promotes inflammation in renal parenchymal cells suffering from ischemia-reperfusion injury. *Cell Death and Disease*, *12*(1), 132. [CrossRef]

Santana, P. T., Benjamim, C. F., Martinez, C. G., Kurtenbach, E., Takiya, C. M., & Coutinho-Silva, R. (2015). The P2X7 receptor contributes to the development of the exacerbated inflammatory response associated with sepsis. *Journal of Innate Immunity*, *7*(4), 417–427. [CrossRef]

Sanz, J. M., & Di Virgilio, F. D. (2000). Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. *Journal of Immunology*, *164*(9), 4893–4898. [CrossRef]

Savio, L. E. B., Andrade, M. G. J., de Andrade Mello, P., Santana, P. T., Moreira-Souza, A. C. A., Kolling, J., Longoni, A., Feldbrügge, L., Wu, Y., Wyse, A. T. S., Robson, S. C., & Coutinho-Silva, R. (2017a). P2X7 receptor signaling contributes to sepsis-associated brain dysfunction. *Molecular Neurobiology*, *54*(8), 6459–6470. [CrossRef]

Savio, L. E. B., de Andrade Mello, P., Figliuolo, V. R., de Avelar Almeida, T. F., Santana, P. T., Oliveira, S. D. S., Silva, C. L. M., Feldbrügge, L., Csizmadia, E., Minshall, R. D., Longhi, M. S., Wu, Y., Robson, S. C., & Coutinho-Silva, R. (2017b). CD39 limits P2X7 receptor inflammatory signaling

and attenuates sepsis-induced liver injury. *Journal of Hepatology*, *67*(4), 716–726. [CrossRef]

Serbina, N. V., Jia, T., Hohl, T. M., & Pamer, E. G. (2008). Monocyte-mediated defense against microbial pathogens. *Annual Review of Immunology*, *26*, 421–452. [CrossRef]

Solle, M., Labasi, J., Perregaux, D. G., Stam, E., Petrushova, N., Koller, B. H., Griffiths, R. J., & Gabel, C. A. (2001). Altered cytokine production in mice lacking P2X7 receptors. *Journal of Biological Chemistry*, *276*(1), 125–132. [CrossRef]

Tancan, E. (2018). *P2x7 reseptörünün beyin elektriksel aktivitesi ve beyin kan akımına olan etkilerının araştırılması*. İstanbul Medipol Üniversitesi Sağlık Bilimleri Enstitüsü.

Trautmann, A. (2009). Extracellular ATP in the immune system: More than just a "danger signal". *Science Signaling*, *2*(56), pe6. [CrossRef]

Turner, C. M., Arulkumaran, N., Singer, M., Unwin, R. J., & Tam, F. W. (2014). Is the inflammasome a potential therapeutic target in renal disease? *BMC Nephrology*, *15*(1), 21. [CrossRef]

Uchino, S., Kellum, J. A., Bellomo, R., Doig, G. S., Morimatsu, H., Morgera, S., Schetz, M., Tan, I., Bouman, C., Macedo, E., Gibney, N., Tolwani, A., Ronco, C., & Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators (2005). Acute renal failure in critically ill patients: A multinational, multicenter study. *JAMA*, *294*(7), 813–818. [CrossRef]

Yan, Y., Bai, J., Zhou, X., Tang, J., Jiang, C., Tolbert, E., Bayliss, G., Gong, R., Zhao, T. C., & Zhuang, S. (2015). P2X7 receptor inhibition protects against ischemic acute kidney injury in mice. *American Journal of Physiology-Cell Physiology*, *308*(6), C463–C472. [CrossRef]

Zhang, Y., Li, F., Wang, L., & Lou, Y. (2021). A438079 affects colorectal cancer cell proliferation, migration, apoptosis, and pyroptosis by inhibiting the P2X7 receptor. *Biochemical and Biophysical Research Communications*, *558*, 147–153. [CrossRef]

Zhang, Y., Yuan, F., Cao, X., Zhai, Z., GangHuang, X., Du, X., Wang, Y., Zhang, J., Huang, Y., Zhao, J., & Hou, W. (2014). P2X7 receptor blockade protects against cisplatin-induced nephrotoxicity in mice by decreasing the activities of inflammasome components, oxidative stress and caspase-3. *Toxicology and Applied Pharmacology*, *281*(1), 1–10. [CrossRef]

Zhu, Y., Li, Q., Xun, W., Chen, Y., Zhang, C., & Sun, S. (2019). Blocking P2X7 receptor ameliorates oxidized LDL-mediated podocyte apoptosis. *Molecular Biology Reports*, *46*(4), 3809–3816. [CrossRef]