Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Adenosine A2A Receptor Activation Alleviated Disease of Mice with Systemic *Candida albicans* Infection by Regulating Macrophage Function

Xia-nan Wu*, Ke Dong*, Yan Liu, Lan Yang, Jing Zhang, Ming Yang, Zhao-wei Gao

Department of Clinical Laboratory, Tangdu Hospital, Airforce Medical University, Xi'an, Shaanxi, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhao-wei Gao, Department of Clinical Laboratory, Tangdu Hospital, Airforce Medical University, Xi'an, Shaanxi, People's Republic of China, Email gaozhaowei1@126.com

Journal of Inflammation Research downloaded from https://www.dovepress.com/
For personal use only.

Purpose: The incidence of candidemia, mediated by systemic *Candida albicans* (*C. albicans*) infection, was increasing. It is an urgent need to understand the underlying disease mechanisms to identify new therapeutic targets. This study aimed to investigate the roles of adenosine–adenosine receptor signal in systemic *C. albicans* infection.

Methods: The candidemia mice models (named CA mice) were established by tail intravenous injection of *C. albicans*. CA Mice were treated with NECA (a metabolically stable adenosine analogue) or agonists targeting different adenosine receptors (A1R, A2AR, A2BR and A3R). The survival rate, renal fungal load and tissue damage were investigated. Bone marrow-derived macrophages (BMDM) were isolated and cultured to investigate the effects of NECA and adenosine receptor agonist on phagocytosis, killing function and polarization of macrophages.

Results: In CA mice, we observed that NECA and A2AR agonist treatment significantly alleviated the sepsis score and increased the survival rate. Moreover, the renal injury and fungal load were reduced by NECA and A2AR agonist treatment. However, the other adenosine receptors (ie, A1R, A2BR and A3R) activation have no effect on survival and tissue damage of CA mice. A2AR activation could reduce macrophage infiltration in kidney and the production of inflammatory cytokine IL-6 in CA mice. Moreover, adenosine-A2AR signaling activation could enhance antifungal capacity of macrophages and promoted macrophage polarization toward the M2 subtype.

Conclusion: Activation of adenosine-A2AR axis promoted macrophage M2 polarization, enhanced host defense against systemic *C. albicans* infection, and alleviated candidiasis. A2AR activation could be considered as a potential therapeutic strategy in candidemia.

Keywords: *C. albicans*, systemic infection, adenosine receptor, renal injury, macrophage

Introduction

Candida species could cause superficial and systemic infection, called candidiasis, which have emerged as a growing threat to humans worldwide, especially in immunocompromised individuals, patients with critically ill or long-term ICU stay or major surgery, etc.[1] Superficial Candida infections affect mucosal surface, which are usually not life threatening, such as vulvovaginal candidiasis and cutaneous candidiasis. Systemic infection (ie, Candidemia) is the most severe infection of candidiasis, which can affect multiple organs including the heart, brain, and kidneys and can potentially lead to septic shock and death.[2] *Candida albicans (C. albicans)* is the predominant pathogen responsible for candidemia, which can invade mucosa barrier and dissemination into the bloodstream, causing sepsis.[3] Due to the high mortality of candidemia, the limitations of antifungal drugs and the emergence of multiple drug-resistant strains,[4] there is great interest in understanding the candidemia mechanism to develop novel therapeutic strategies.

Received: 29 October 2024
Accepted: 22 February 2025
Published: 6 March 2025

© 2025 Wu et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

The immune response to infection must be appropriate to provide adequate effects in clearance of pathogens while avoiding overheated inflammation, which could induce extensive tissue damage. Therefore, both pro- and anti-inflammatory factors play important roles during infection. Especially in the later stages of infection, the control of inflammation is crucial for remission of the disease.

Adenosine is an "alarm signal" under stress condition, such as hypoxia, injury, infection, mediating immunosuppressive effects and aiming to restore internal homeostasis.[5] There were four adenosine receptors, ie, A1R, A2AR, A2BR and A3R. Adenosine activates adenosine receptors to trigger signaling cascades resulting in inhibiting inflammatory responses. Notably, adenosine-receptor signaling is involved in pathogen infection, host immune response and inflammation regulation processes, showing both aggravating and alleviating effects in infectious diseases. Nascimento DC et al's study showed that sepsis results in elevated adenosine in circulation and extracellular adenosine triggers immunosuppressive signaling via the A2AR.[6] In polymicrobial sepsis, inhibiting A2AR signaling could boost survival by enhancing the pathogene clearance.[7] In polymicrobial sepsis mice models, A2AR knockout could reduce the sepsis score and increase the survival rate of mice models.[8] Conversely, Schingnitz U et al showed that A2BR activation resulted in attenuation of lung inflammation and pulmonary edema in sepsis mice.[9] Gallos G and colleagues' studies demonstrated that the A1R and A3R activation confer a protective effect in mice from septic peritonitis primarily by attenuating the hyperacute inflammatory response in sepsis.[10,11] Ngamsri KC's report also showed the protective effect of A2BR in murine peritonitis-induced sepsis models.[12] However, the function of adenosine-receptor signaling in *C. albicans* infection was much less known.

In this study, we established a mouse model of *C. albicans* systemic infection (ie, CA mice) and found that serum adenosine levels were significantly decreased after *C. albicans* infection. Subsequently, we investigated the effects of NECA (a metabolically stable adenosine analogue) and agonists of different adenosine receptors CCPA, CGS21680, BAY60-6583, and 2-CI-IB-MECA (targeting A1R, A2AR, A2BR, and A3R, respectively) on CA mice. Moreover, we showed that NECA treatment could improve the survival of CA mice and alleviated the renal injury through activating A2AR. Furthermore, we investigated the effect of adenosine-A2AR signal on the function of macrophage. In conclusion, our study presents novel therapeutic approaches for *C. albicans* systemic infections.

Materials and Methods

Mice

C57BL/6J mice (female, 6–8 weeks, 18–20g) were purchased from the animal center of Air Force Medical University. The mice were fed under specific pathogen-free condition and utilized in accordance with animal welfare guidelines, which approved by the Institutional Animal Care and Use Committee of Air Force Medical University (No. 20241353). All animal experiments are conducted in accordance with the US Public Health Service Policy on the Use of Laboratory Animals.[13]

Candida albicans Culture

The *C. albicans* standard strain ATCC90028 was cultured on yeast extract, peptone, and dextrose (YPD) agar plates for 48 hours to isolate single colonies. Following two serial passages, *C. albicans* were collected through centrifugation, rinsed twice with sterile phosphate buffered saline (PBS), and subsequently re-suspended in PBS for further experiment.

Mice Models of Systemic Candidiasis

CA group: Mice were infected intravenously with 2.0×10^5 colony forming units (CFU) *C. albicans* via the lateral tail vein. Control group: The normal mice were intravenously with PBS. Intervention group: CA mice were intraperitoneal injection with adenosine analogues (NECA; 50 μg/kg, Cat: GC15304, GLPBIO, USA), A1R agonist (CCPA; 1mg/kg, Cat: GC14369, GLPBIO, USA), A2AR agonist (GCS21680; 1mg/kg, Cat: GC10172; GLPBIO, USA) or antagonist (KW6002; 2mg/kg, Cat: GC11590, GLPBIO, USA), A2BR agonist (BAY 60–6583; 4mg/kg, Cat: HY-103171, MedChemExpress, USA) and A3R agonist (2-CI-IB-MECA; 0.2m/kg, Cat: HY-12365, MedChemExpress, USA). The mice were observed daily for listlessness, crouching, eye closure, hair standing and slow activity, and the weight loss and

survival of the mice were recorded after infection. As described previously,[14] the Mouse Clinical Assessment Score for Sepsis (M-CASS) were determined according to six indexes: fur aspect, activity, posture, behavior, chest movements, eyelids. The mice models were scored from 0 to 3.

Detection of Fungal Burden in Murine Kidney Tissue

After 7 days of infection, mice kidney tissue was collected, weighed and homogenized. After a series of dilutions, tissue homogenate was evenly coated on YPD plates and incubated at 37 °C for 36–48 hours. The fungal burdens were represented by CFU per gram of kidney tissue.

Histopathology and Immunohistochemistry (IHC) Analysis

The kidneys from infected mice were removed and fixed in 4% Paraformaldehyde solution, embedded in paraffin, and sectioned them into 3–5μm thick. These sections were stained with haematoxylin and eosin (H&E) or periodic-acid-Schiff (PAS). Pannoramic and CaseViewer 2.4 software (3DHISTECH, Hungary) were used for image acquisition.

IHC assay was used to evaluate the protein levels in kidney tissue. Briefly, tissue sections were deparaffinized in xylene, rehydrated through graded ethanol, followed by blocking of endogenous peroxidase activity in 3% hydrogen peroxide for 25 min at room temperature. The anti-F4/80 antibody (1:1000, Cat: GB113373, Servicebio, Wuhan, China) and anti-LY6G antibody (1:1000, Cat: GB11229, Servicebio, Wuhan, China) were used as the primary antibodies. The sheep anti-rabbit (1:200 dilution, Cat: GB23303, Servicebio, Wuhan, China) and sheep anti-mouse (1:200 dilution, Cat: GB2330, Servicebio, Wuhan, China) were used as the second antibody. The IHC scores calculated by using the multiply of staining intensity and the percentage of positive staining cells.[15] Staining intensity was evaluated by four degrees: 0=negative; 1=weak positive; 2=positive; 3=strong positive. The percentage of positive cells was given into four grades: 1 for <25%; 2 for 25–50%; 3 for 50–75%; 4 for 75–100%. Each section was assayed for 10 independent high magnifications (×400) fields.

Measurement of Urea Nitrogen, Uric Acid and Creatinine

Blood was collected from anesthetized mice via cardiac puncture. The serum was separated by centrifugation and stored at −80°C. The serum levels of urea nitrogen (BUN), uric acid (UA) and creatinine (CR) were analyzed using a automatic biochemical analyzer (Beckman Coulter, AU5800, USA).

Measurement of IL-6

The kidney tissue was homogenized in PBS and centrifuged to harvest the supernatant. IL-6 levels in serum and supernatant of mice were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocols (Cat: VAL604G, Novus, USA).

Measurement of Adenosine

Serum adenosine levels were detected by high-performance liquid chromatography-mass spectrometry (LC-MS; Shimadzu, Kyoto, Japan), operating in positive mode (ESI^+). The separations were carried out using a 100 × 2.1 mm Shim-pack GIST-HP column with a particle size of 3.5 μm (Shimadzu, Kyoto, Japan). The mobile phase consisted of 0.1% formic acid in water and 0.1% formic acid in methanol. The flow rate was set to 0.2 mL/min, the column temperature was 40°C, and the injection volume was 2 μL. The ions detection was performed in the multiple reaction monitoring (MRM) mode, monitoring the transitions of the m/z 268 precursor ion to the m/z 136 production for adenosine (268 > 136) and 230 > 112 for lamivudine (internal standard). The analytical data were processed by the labsolution 5.81 (Shimadzu, Kyoto, Japan).

Isolation and Culture of Bone Marrow Derived Macrophages (BMDM)

Bone marrow cells were harvested from femur and tibia of mice by rinsing repeatedly with Dulbecco's Modified Eagle Medium (DMEM). The cells were filtered using a 70 μm filter, added into the red blood cell lysate, then centrifuged. The cells were resuspended in DMEM containing 10% FBS (ExCellBio, Shanghai, China), 100 U/mL penicillin, 100 mg/mL

streptomycin (Hyclone, Logan, USA) and 30 ng/mL mouse M-CSF (cat: 51112-MNAH, Sino Biological Inc., Beijing, China), and incubated for 5 days at 37°C with 5% CO_2. After 5–6 days of culturing, flow cytometry (FCM) analysis showed that more than 80% of cells were F4/80$^+$ macrophages.

Macrophage Fungal Phagocytosis Assay

The phagocytosis assay of heat-killed *C. albicans* (HKCAs) was conducted as described previously with minor modification.[16] Briefly, HKCAs were labeled with FITC (Cat: ST2065, Beyotime, Shanghai, China) in PBS buffer for 30 minutes. Subsequently, BMDM were co-cultured with FITC-labeled HKCAs (MOI=1:5) at 37°C for 45 minutes. The cell nucleus was stained with DAPI (Cat: C1005, Beyotime, Shanghai, China) for 10 minutes and photographed by using a fluorescence microscope (Olympus, Japan). Phagocytosis was expressed as the percentage of phagocytosing FITC-labeled *C. albicans*.

Macrophage Fungal Killing Assay

To test fungicidal capacity, 2×10^5 macrophages were seeded in 12-well plates and incubated overnight at 37°C in a humidified 5% CO_2 incubator. Macrophage fungal killing assay was performed as described previously.[17,18] Macrophages were treated with NECA (10μM) or CGS21680 (10μM) for 1 hour before co-culture with *C. albicans*. After 3 hours of incubation with *C. albicans* (MOI=1:1), the macrophages were then disrupted with 0.05% Triton-X-100, washed twice with PBS and coated on YPD plates. Colonies were counted after 36–48 h at 37°C. Fungicidal capacity was defined as [1- (number of uningested CFU/CFU at the start of incubation)] × 100%.

Flow Cytometry

The collected cells were incubated with fluorochrome-conjugated antibodies for 30 min at room temperature away from light. [Antibodies: F4/80-PE (Cat: FMP480, 4A Biotech Co., Ltd, Beijing, China), CD86-FITC (Cat: FMF086, 4A Biotech Co., Ltd, Beijing, China) and CD206-FITC (Cat: 141703, Biolegend, US)]. Then, cells were washed twice and resuspended with PBS. Fluorescent signals were quantitated by flow cytometer (Agilent Novocyte, Hangzhou, China).

Real-Time Quantitative PCR

Total RNA was extracted using 1 mL of TRIzol (Takara, Japan) according to the manufacturer's protocol. mRNA was reverse transcribed into cDNA using the PrimeScript RT Reagent (Cat: AG21102, Accurate Biotechnology, Hunan, China). Quantitative PCR was performed with a SYBR Green PCR kit (Abm, Jiangsu, China) using gene-specific primers (Supplementary Table S1). The relative expression was calculated as $2^{-\Delta\Delta Ct}$ normalized to the housekeeping gene GAPDH.

Statistical Analysis

Normally distributed data were presented as mean ± standard deviation (SD) and analyzed by Two-tailed Student's *t*-test, one-way ANOVA with a Bartlett's multiple comparison test and 2-way ANOVA with Dunnette's multiple comparison tests. Non-normally distributed data were as median (interquartile range, IQR) and analyzed by Mann–Whitney *U*-tests or Kruskal–Wallis tests with Dunn's multiple comparison tests. Log rank tests were used for survival analysis. Data were plotted using GraphPad Prism software. $P < 0.05$ was considered as statistically significant.

Results

Adenosine Alleviated Disease in CA Mice

By using CA mice models, we first detected serum levels of adenosine. The liquid chromatography-mass spectrometry (LC-MS) results showed that the serum adenosine level of CA mice was significantly decreased compared with the control group (Figure 1A). Subsequently, we investgated the effect of NECA (a metabolically stable adenosine analogue) on CA disease. The procedure of mice experiments is shown in Figure 1B. We observed that the survival rate of mice was significantly improved in the NECA treatment group compared with CA mice (Figure 1C). Accordingly, the weight

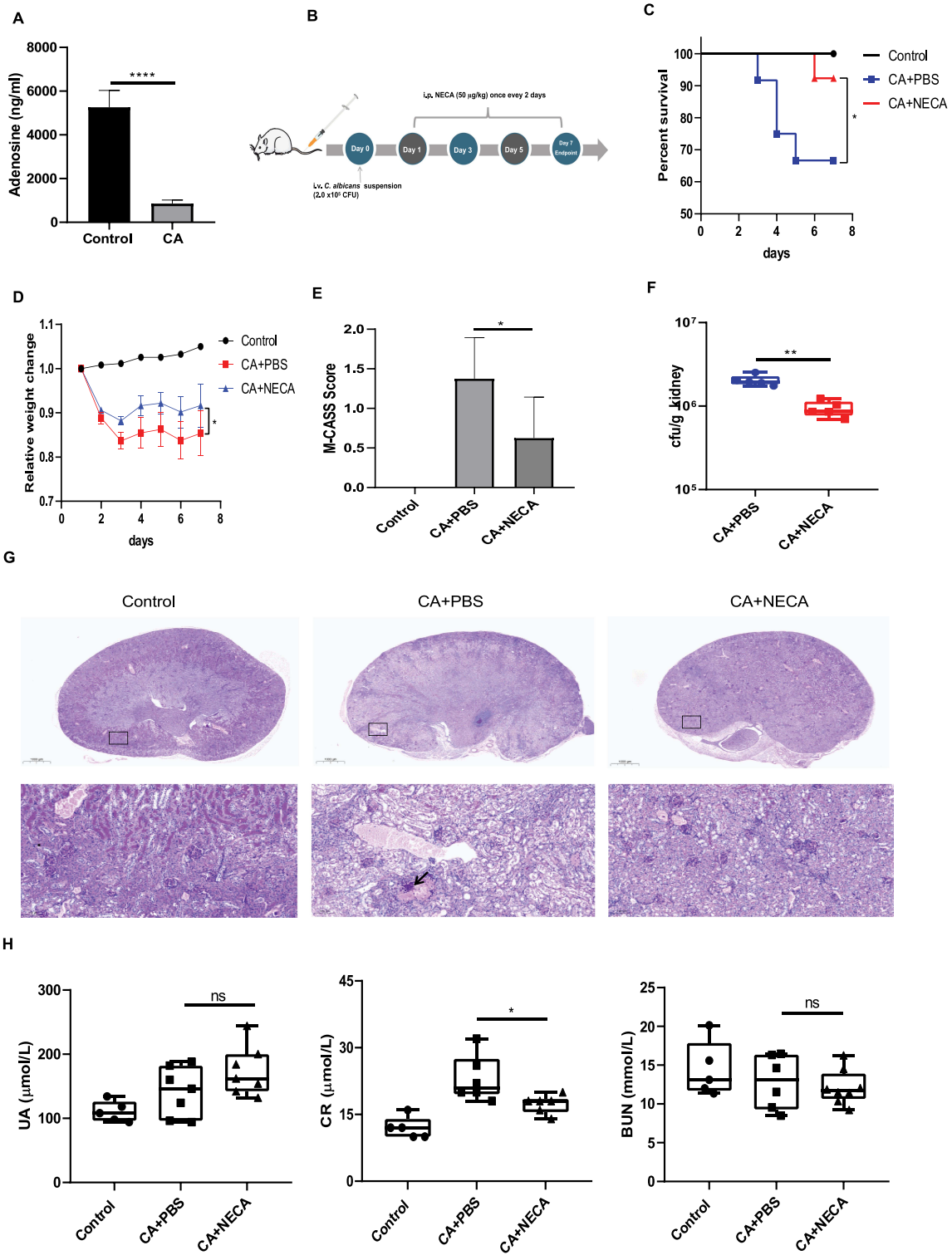

Figure 1 Adenosine alleviated disease in systemic *C. albicans* infection. **(A)** Adenosine in mouse serum was quantified by LC-MS (liquid chromatography-mass spectrometry). Serum adenosine levels were significantly reduced in mice infected with *C. albicans*. ****, $P<0.0001$, compared with the control group. **(B)** The flow diagram of mice experiments. The normal control mice were intravenously with saline (ie, Control group). A model group was constructed by infecting mice with 2×10^5 CFU *C. albicans* intravenously (named CA group), and an equal amount of PBS was injected as a CA+PBS group. CA mice were intraperitoneal with adenosine analogues NECA (named CA + NECA group). **(C** and **D)** The survival rate and weight loss of mice in the NECA group. **(E)** The Mouse Clinical Assessment Score for Sepsis (M-CASS) scores in the NECA group. **(F)** The fungal burden of kidney in the NECA group. **(G)** Periodic-acid-Schiff (PAS) staining of the kidney lesion in NCEA treated mice. The black arrows represent oval spores and pseudomycelium. Scale bar = 1000 μm; scale bar = 100 μm. **(H)** The serum levels of renal function markers, including blood urea nitrogen (BUN), uric acid (UA) and creatinine (CR), were detected in the NECA group. *and **, $P<0.05$ and $P<0.01$, respectively, compared with the CA+PBS group.

https://doi.org/10.2147/JIR.S501546

loss of mice was significantly reduced (Figure 1D), and the Mouse Clinical Assessment Score for Sepsis (M-CASS) scores were significantly decreased in the NECA group (Figure 1E). The fungal burden was significantly lower in the NECA group than CA mice (Figure 1F). Consistently, Periodic-acid-Schiff (PAS) staining showed that the number of purplish red ovoid spore and pseudomycelium was significantly reduced in the kidney lesion of NCEA treated mice (Figure 1G). Serological tests showed that serum creatinine (CR) levels were significantly down-regulated in the NECA group (Figure 1H).

A2AR Agonist Improves Survival and Renal Function in CA Mice

To explore the potential mechanism of adenosine in CA mice, we investigated the effect of adenosine receptors (ie, A1R, A2AR, A2BR and A3R) agonist on disease of CA mice. The procedure of mice experiments is shown in Figure 2A. We observed that the survival rate was significantly higher in A2AR agonist (CGS21680) treated group compared to the vehicle group (ie, DMSO treated CA mice) (Figure 2B). However, there were no significant effect of A1R/A2BR/A3R agonist on survival of CA mice (Figure 2C-E). Consistently, A2AR antagonist (KW6002) treatment could lead to more deaths in CA mice (Figure 2F). Moreover, the M-CASS score and kidney fungal burden were significantly reduced in A2AR agonist group (vs CA+DMSO group), while they were increased in A2AR antagonist group (Figure 2G and H). PAS staining also showed a decrease in oval spores and pseudomycelium in kidney tissue of A2AR agonist group (Figure 2I). Renal function injury index (BUN and CR) was significantly decreased in A2AR agonist group (Figure 2J). Taken together, these results demonstrated that A2AR activation could alleviate CA disease and improve survival. Notably, there were no significant effect of other adenosine receptors (ie, A1R, A2BR and A3R) on CA mice's disease scores, fungal burden and renal function (Supplementary Figures S1-S2).

A2AR Agonist Inhibited Pathological Damage and Inflammatory in Kidney of CA Mice

Haematoxylin and eosin (H&E) staining and immunohistochemical (IHC) analysis were used to further evaluate the infiltration of inflammatory cells in the kidneys of CA mice. As shown in Figure 3A, in kidney from CA mice (vs control mice), there were multifocal necrosis, reduced glomerular number and uneven distribution. Moreover, a large number of tubular epithelial was necrotic and inflammatory cells were found in the lumen. The A2AR agonist treatment significantly alleviated renal pathological injury and inflammation, and A2AR antagonist treatment aggravated renal pathological injury. IHC results showed that $F4/80^{+}$ macrophage infiltration in kidney were decreased in A2AR agonist treated CA mice (Figure 3B). Moreover, the IL-6 levels in serum and kidney were significantly decreased in A2AR agonist treatment group and increased in A2AR antagonist treatment group (Figure 3C and D). Taken together, these data indicated that A2AR activation reduced the macrophages number and inflammatory cytokines production in CA mice kidney.

A2AR Agonist Promoted M2 Polarization of Macrophages in CA Mice

We further investigated the effect of NECA and CGS21680 on macrophages. First, we observed that the increased phagocytosis efficiency targeting *C. albicans* of macrophage by NECA and CGS21680 treatment (Figure 4A). Notably, the killing efficiency against *C. albicans* of NECA and CGS21680 treated macrophage was significantly increased ($P < 0.05$; Figure 4B). Second, NCEA and CGS21680 treatment significantly promoted the polarization of macrophages shifted towards M2. After NECA and CGS21680 treatment, FCM analysis showed that the $CD86^{+}$ M1 macrophage cell ratio was down-regulated, while the $CD206^{+}$ M2 macrophage cell ratio was up-regulated (Figure 4C and D). Accordingly, the qRT-PCR showed that the expression of M1 markers was significantly down-regulated, including CD86, IL-6 and iNOS, while the expression of M2 markers was up-regulated, including CD206, ARG-1, CD163 (Figure 4E and F). Taken together, these results demonstrated that activation of adenosine-A2AR axis promoted macrophage polarization to the M2 types.

Discussion

The early toxemia of *C. albicans* sepsis is mild, easy to be covered by the primary disease and secondary infection. However, multiple organ failure or septic shock may occur in critically ill CA patients.[3,19] Given the high infection-

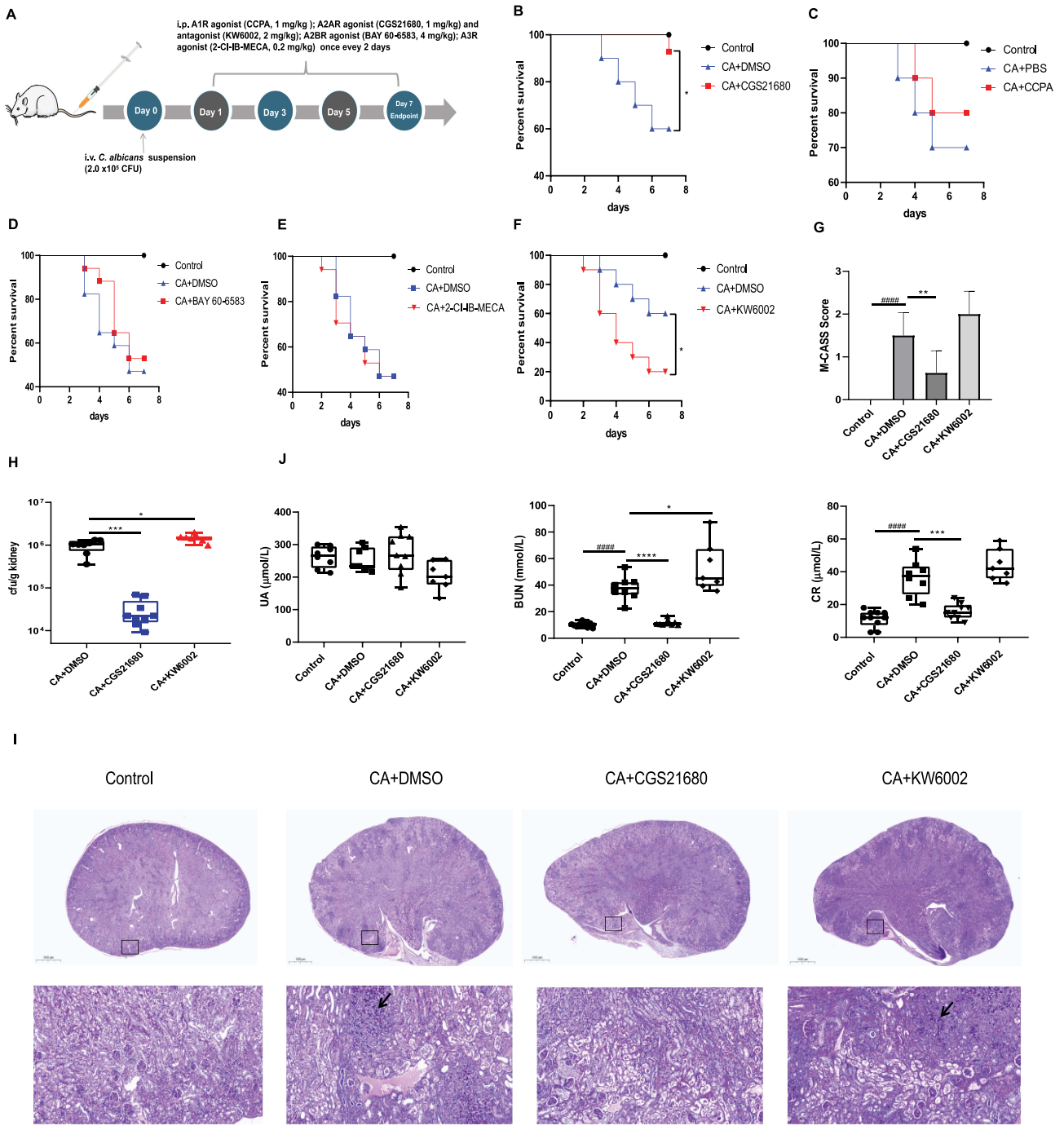

Figure 2 A2AR agonist improves survival and enhances fungal clearance in invasive *C. albicans* infection. **(A)** The procedure of mice experiments. Intervention group: CA mice were interpertional with A1R agonist (CCPA), A2AR agonist (GCS21680) or antagonist (KW6002), A2BR agonist (BAY 60–6583) and A3R agonist (2-Cl-IB-MECA). **(B)** The survival rate of A2AR agonist (CGS21680) and vehicle group (ie, DMSO treated CA mice). **(C-E)** The effect of A1R/A2BR/A3R agonist on survival of CA mice. **(F)** The survival of A2AR antagonist (KW6002) in CA mice. (**G** and **H**) The M-CASS scores and kidney fungal burden of CGS21680 and KW6002 group in CA mice. **(I)** Representative mouse kidney pictures with PAS staining in each group. As indicated by the black arrow in the picture, the spores and pseudomycelium of the fungus appear purplish red. Scale bar = 1000 μm; scale bar = 100 μm. **(J)** Renal function injury index (UA, BUN and CR) in CGS21680 and KW6002 group. *, **, *** and ****, $P<0.05$, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively, compared with the CA+DMSO group. #####, $P<0.0001$, compared with the control group.

associated mortality and limited antifungal drug options, the search for reliable therapeutic target is important to reduce mortality in patients with *C. albicans* sepsis.

Adenosine, as an immune-suppression signal, plays a crucial role in the inflammation regulation during stress, such as hypoxia, tissue damage and infection. In this study, we constructed a mouse model of invasive *C. albicans* infection, and

https://doi.org/10.2147/JIR.S501546

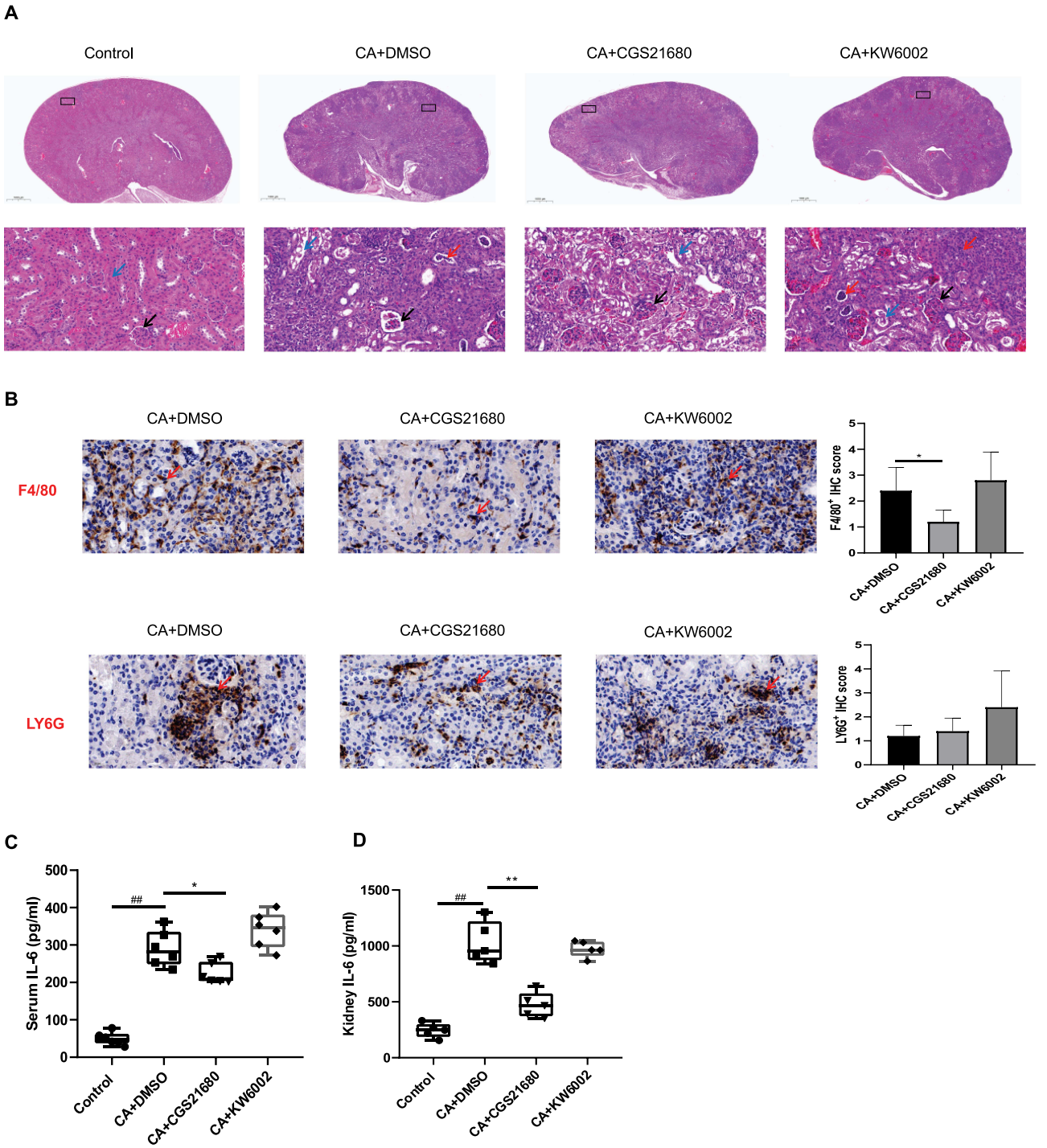

Figure 3 Adenosine-A2AR signal inhibits renal inflammation in mice infected with *C. albicans*. **(A)** Haematoxylin and eosin (HE) staining was used to evaluate renal inflammatory infiltration in mice. As is shown in the picture, the black arrows represent the glomeruli, the blue arrows represent the renal tubule cuticle cells, and the red arrows represent infiltrating inflammatory cells. **(B)** The recruitment of mouse renal macrophages and neutrophils was assessed by immunohistochemical (IHC) staining in infected mice. Positive stained areas are indicated by red arrows. Scale bar = 20μm. The IHC score results were semi-quantitatively evaluated by the multiply of staining intensity and the percentage of positive staining cells. (**C** and **D**) IL-6 levels in serum and kidney homogenate supernatant of infected mice were detected by enzyme-linked immunosorbent assay (ELISA). *, and **, $P<0.05$ and $P<0.01$, respectively, compared with the CA+DMSO group. ##, $P<0.01$, compared with the control group.

found that the serum adenosine level of mice was significantly decreased. Subsequently, we observed a significant improved survival in NECA (adenosine analogue) treated CA mice (vs vehicle treated CA mice). Although *C. albicans* can be detected in the brain, lungs, and kidneys after entering the bloodstream, pathological damage and fungal burden

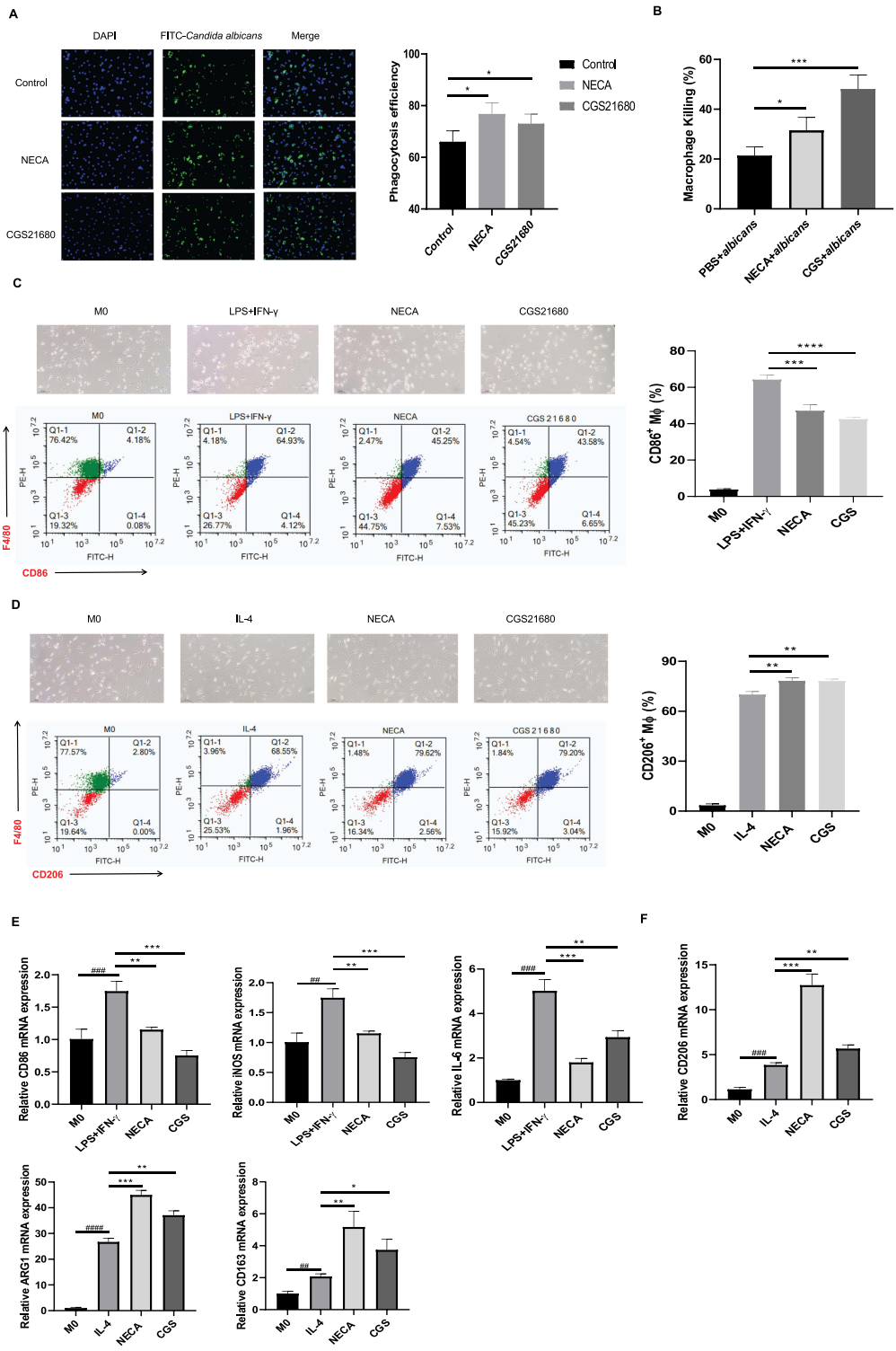

Figure 4 A2AR agonist promoted M2 polarization of macrophages in CA mice. **(A)** Bone marrow-derived macrophages (BMDM) were co-cultured with *C. albicans* for phagocytosis and killing in vitro. Phagocytosis efficiency of macrophages treated with NECA and CGS21680 against heat-inactivated FITC-labeled *C. albicans* (MOI=1:5). *, $P<0.05$, compared with the control group. **(B)** The killing ability of macrophages treated with NECA and CGS21680 against *C. albicans* (MOI=1:1). * and ***, $P<0.05$ and $P<0.001$, respectively, compared with the PBS+ *albicans* group. **(C)** Representative pictures of the M1 polarization of macrophage, with initial M0 macrophages as negative controls, LPS- and interferon γ (IFN-γ)-stimulated macrophages as positive controls. The percentage of M1 macrophages ($F4/80^+CD86^+$) in NECA and CGS21680 treatment groups was analyzed by flow cytometry. *** and ****, $P<0.001$ and $P<0.0001$, respectively, compared with the LPS+ IFN-γ group. **(D)** Representative pictures of the M2 polarization of macrophage, with initial M0 macrophages as negative controls, IL-4-stimulated macrophages as positive controls. The percentage of M2 macrophages ($F4/80^+CD206^+$) in NECA and CGS21680 treatment groups was determined by flow cytometry. **, $P<0.01$, compared with the IL-4 group. **(E)** The expression of M1 markers was detected by RT-qPCR. ** and ***, $P<0.01$ and $P<0.001$, respectively, compared with the LPS+ IFN-γ group. **(F)** The expression of M2 markers in each group of macrophage was evaluated by RT-qPCR. *, ** and ***, $P<0.05$, $P<0.01$ and $P<0.001$, respectively, compared with the IL-4 group. ##,### and #####, $P<0.01$, $P<0.001$ and $P<0.0001$, respectively, compared with the M0 group.

https://doi.org/10.2147/JIR.S501546

are more severe in the kidney tissue.[16,20] Li M et al emphasized that the kidney is the main affected organ during systemic *candida* infection, which may be related to the abundant blood flow in the kidney.[21] Thus, renal injury is an important index to evaluate the severity of candidiasis. In this study, PAS staining showed that the number of fungal spores and pseudomycelia was significantly reduced in the kidney section from NECA treated CA mice (vs vehicle treated CA mice). In addition, serum creatinine also decreased significantly after NECA treatment. These data suggest that adenosine could protect the host from grievous injury during *C. albicans* infection.

Adenosine receptors (A1R, A2AR, A2BR and A3R) are widely expressed on cell membranes. There were significant different function among these four adenosine receptors, which were involved in different biological processes.[22,23] To further explore the mechanism of adenosine during *C. albicans* infection, we treated CA mice with A1R/A2AR/A2BR/A3R agonists respectively. We observed that A2AR activation could alleviated the renal damage and inflammatory and improve the survival of CA mice. Consistently, we further treated CA mice with A2AR antagonists, and the renal fungal burden and pathological damage were more severe. However, activation of the other three adenosine receptors (ie, A1R, A2BR, A3R) has no significant effect on survival and renal injury of CA mice. Previous studies have shown that adenosine-A2AR signaling plays an important role in kidney injury and repair. Using immune nephritis models, Truong LD et al found that A2AR receptor deficiency mice had higher levels of pro-inflammatory cytokines, more accumulation of inflammatory cells, and more severe kidney damage.[24] Huang CC et al highlighted that the activation of A2AR can improve renal dysfunction in cirrhotic rats.[25] Taken together, our data demonstrated that adenosine-A2AR axis was involved in CA progress, especially in CA-related renal damage.

The innate immunity mediated by macrophages is an important mechanism in host defense against *C. albicans* infection.[26,27] M1 and M2 subtypes of macrophage play different roles in human immune response.[28] A2AR receptor is present in macrophages, and its mediated adenosine signaling affects the production of inflammatory cytokines and microbial killing by macrophages.[6,29,30] By immunohistochemical analysis, we found that A2AR receptor agonists could reduce macrophage infiltration in the kidneys of infected mice. CGS21680 treatment significantly decreased the level of IL-6 in serum and kidney homogenate supernatant. The cell killing experiment showed that NECA or CGS21680 could significantly improve the antimicrobial activity of macrophages against *C. albicans*. Dos Santos PMF et al have shown that A2AR agonists can decrease the production of cytokines IL-6 and IL-8 and reduce intracellular *M. leprae* viability in human cells.[31] Mou KJ et al reported that adenosine-A2AR activation-induced macrophages M2 polarization, increased the expression of anti-inflammatory factor IL-10 and suppressed the expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β).[32] Consistently, FCM analysis showed that treatment of BMDM with NECA and A2AR agonists inhibited M1 polarization and promoted M2 polarization. These results demonstrated that adenosine-A2AR axis allviated CA disease might be via promoting the antimicrobial activity and M2 polarization of macrophages. Thus, Adenosine-A2AR receptor signaling is expected to be a potential therapeutic strategy for candidiasis. Considering that adenosine-A2AR axis intervention can affect immune homeostasis, the side effects of immunosuppression still need to be carefully studied.

Conclusion

In conclusion, adenosine-A2AR axis activation improves the survival rate of mice infected with *C. albicans*, inhibits the inflammatory response and kidney damage. These findings demonstrated an important mechanism and therapeutic target of progression of invasive *candida* infections. Future studies may further explore the role of A2AR receptor in the immune escape mechanism of *candida* infection. In addition, the development of drugs targeting A2AR receptors will also become an important direction in the field of candidiasis treatment. Through the development of highly effective and specific A2AR receptor agonists, combined with anti-fungal drugs and other combination therapies, it is expected to achieve accurate treatment of *candida* infection and improve the survival rate and quality of life of patients.

Acknowledgments

This work was supported by the Fund of Tangdu Hospital (No.2021SHRC059).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Lass-Flörl C, Kanj SS, Govender NP, et al. Invasive candidiasis. *Nat Rev Dis Primers*. 2024;10(1):20. doi:10.1038/s41572-024-00503-3
2. Katsipoulaki M, Stappers MHT, Malavia-Jones D, et al. Candida albicans and Candida glabrata: global priority pathogens. *Microbiol Mol Biol Rev*. 2024;88(2):e0002123. doi:10.1128/mmbr.00021-23
3. Logan C, Martin-Loeches I, Bicanic T. Invasive candidiasis in critical care: challenges and future directions. *Intensive Care Med*. 2020;46 (11):2001–2014. doi:10.1007/s00134-020-06240-x
4. Fang W, Wu J, Cheng M, et al. Diagnosis of invasive fungal infections: challenges and recent developments. *J Biomed Sci*. 2023;30(1):42. doi:10.1186/s12929-023-00926-2
5. Allard B, Allard D, Buisseret L, et al. The adenosine pathway in immuno-oncology. *Nat Rev Clin Oncol*. 2020;17(10):611–629. doi:10.1038/s41571-020-0382-2
6. Nascimento DC, Viacava PR, Ferreira RG, et al. Sepsis expands a CD39+ plasmablast population that promotes immunosuppression via adenosine-mediated inhibition of macrophage antimicrobial activity. *Immunity*. 2021;54(9):2024–2041.e8. doi:10.1016/j.immuni.2021.08.005
7. Németh ZH, Csóka B, Wilmanski J, et al. Adenosine A2A receptor inactivation increases survival in polymicrobial sepsis. *J Immunol*. 2006;176 (9):5616–5626. doi:10.4049/jimmunol.176.9.5616
8. Meriño M, Martín SS, Sandaña P, et al. Deletion of the adenosine A2A receptor increases the survival rate in a mice model of polymicrobial sepsis. *Purinergic Sig*. 2020;16(3):427–437. doi:10.1007/s11302-020-09719-w
9. Schingnitz U, Hartmann K, Macmanus CF, et al. Signaling through the A2B adenosine receptor dampens endotoxin-induced acute lung injury. *J Immunol*. 2010;184(9):5271–5279. doi:10.4049/jimmunol.0903035
10. Gallos G, Ruyle TD, Emala CW, et al. A1 adenosine receptor knockout mice exhibit increased mortality, renal dysfunction, and hepatic injury in murine septic peritonitis. *Am J Physiol Renal Physiol*. 2005;289(2):F369–76. doi:10.1152/ajprenal.00470.2004
11. Lee HT, Kim M, Joo JD, et al. A3 adenosine receptor activation decreases mortality and renal and hepatic injury in murine septic peritonitis. *Am J Physiol Regul Integr Comp Physiol*. 2006;291(4):R959–69. doi:10.1152/ajpregu.00034.2006
12. Ngamsri KC, Fabian F, Fuhr A, et al. Sevoflurane exerts protective effects in murine peritonitis-induced sepsis via hypoxia-inducible factor 1α/adenosine A2B receptor signaling. *Anesthesiology*. 2021;135(1):136–150. doi:10.1097/ALN.0000000000003788
13. National Institutes of Health (US). Laboratory animal welfare: public health service policy on humane care and use of laboratory animals by awardee institutions; notice. *Fed Regist*. 1985;50(90):19584–19585.
14. Mai SHC, Sharma N, Kwong AC, et al. Body temperature and mouse scoring systems as surrogate markers of death in cecal ligation and puncture sepsis. *Intensive Care Med Exp*. 2018;6(1):20. doi:10.1186/s40635-018-0184-3
15. Xu Q, Liu X, Liu Z, et al. MicroRNA-1296 inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting SRPK1-mediated PI3K/AKT pathway. *mol Cancer*. 2017;16(1):103. doi:10.1186/s12943-017-0675-y
16. Liu J, Lai X, Yu R, et al. Progranulin aggravates lethal Candida albicans sepsis by regulating inflammatory response and antifungal immunity. *PLoS Pathog*. 2022;18(9):e1010873. doi:10.1371/journal.ppat.1010873
17. Desai JV, Kumar D, Freiwald T, et al. C5a-licensed phagocytes drive sterilizing immunity during systemic fungal infection. *Cell*. 2023;186 (13):2802–2822.e22. doi:10.1016/j.cell.2023.04.031
18. Chen Y, Jiang Q, Qing F, et al. MDA5 enhances invasive Candida albicans infection by regulating macrophage apoptosis and phagocytosis/killing functions. *Inflammation*. 2024;47(1):191–208. doi:10.1007/s10753-023-01903-5
19. McCarty TP, White CM, Pappas PG. Candidemia and invasive candidiasis. *Infect Dis Clin North Am*. 2021;35(2):389–413. doi:10.1016/j.idc.2021.03.007
20. Jiang Q, Chen Y, Zheng S, et al. AIM2 enhances Candida albicans infection through promoting macrophage apoptosis via AKT signaling. *Cell Mol Life Sci*. 2024;81(1):280. doi:10.1007/s00018-024-05326-9
21. Li M, Li C, Wu X, et al. Microbiota-driven interleukin-17 production provides immune protection against invasive candidiasis. *Crit Care*. 2020;24 (1):268. doi:10.1186/s13054-020-02977-5
22. Olah ME, Stiles GL. Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu Rev Pharmacol Toxicol*. 1995;35(1):581–606. doi:10.1146/annurev.pa.35.040195.003053
23. Gomez-Castro F, Zappettini S, Pressey JC, et al. Convergence of adenosine and GABA signaling for synapse stabilization during development. *Science*. 2021;374(6568):eabk2055. doi:10.1126/science.abk2055
24. Truong LD, Trostel J, McMahan R, et al. Macrophage A2A adenosine receptors are essential to protect from progressive kidney injury. *Am J Pathol*. 2016;186(10):2601–2613. doi:10.1016/j.ajpath.2016.06.017
25. Huang CC, Shen HC, Lin R, et al. Preservation of vascular endothelial glycocalyx and barrier by activation of adenosine A2A receptor (A2AR) improved renal dysfunction in cirrhotic rats. *J Pharmacol Sci*. 2023;152(1):50–60. doi:10.1016/j.jphs.2023.03.002
26. Ding X, Kambara H, Guo R, et al. Inflammasome-mediated GSDMD activation facilitates escape of Candida albicans from macrophages. *Nat Commun*. 2021;12(1):6699. doi:10.1038/s41467-021-27034-9
27. Qing F, Sui L, He W, et al. IRF7 exacerbates Candida albicans infection by compromising CD209-mediated phagocytosis and autophagy-mediated killing in macrophages. *J Immunol*. 2024;212(12):1932–1944. doi:10.4049/jimmunol.2300826
28. Chen X, Liu Y, Gao Y, et al. The roles of macrophage polarization in the host immune response to sepsis. *Int Immunopharmacol*. 2021;96:107791. doi:10.1016/j.intimp.2021.107791
29. Haskó G, Pacher P. Regulation of macrophage function by adenosine. *Arterioscler Thromb Vasc Biol*. 2012;32(4):865–869. doi:10.1161/ATVBAHA.111.226852
30. Strakhova R, Cadassou O, Cros-Perrial E, et al. Regulation of tumor infiltrated innate immune cells by adenosine. *Purinergic Sig*. 2020;16 (3):289–295. doi:10.1007/s11302-020-09701-6
31. Dos Santos PMF, Díaz Acosta CC, Tlsa R, et al. Adenosine A2A receptor as a potential regulator of Mycobacterium leprae survival mechanisms: new insights into leprosy neural damage. *Front Pharmacol*. 2024;15:1399363. doi:10.3389/fphar.2024.1399363
32. Mou KJ, Shen KF, Li YL, et al. Adenosine A2A receptor in bone marrow-derived cells mediated macrophages M2 polarization via PPARγ-P65 pathway in chronic hypoperfusion situation. *Front Aging Neurosci*. 2022;13:792733. doi:10.3389/fnagi.2021.792733

Journal of Inflammation Research

Dovepress
Taylor & Francis Group

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/journal-of-inflammation-research-journal