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$A_{2A}R$ regulate inflammation through PKA/NF-κB signaling pathways in intervertebral disc degeneration

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Abstract

Background Reduction of inflammatory damage and inhibition of nucleus pulposus (NP) apoptosis are considered to be the main effective therapy idea to reverse the intervertebral disc degeneration (IDD) and alleviate the chronic low back pain. The adenosine A2A receptor (A2AR), as a member of G protein-coupled receptor families, plays an important role in the anti-inflammation and relieving pain. So far, the impact of A2AR on IDD therapy is unclear. The aim of this study was to explore the role of Adenosine A_{2A} receptor ($A_{2A}R$) in the intervertebral disc degeneration (IDD) and clarify potential mechanism.

Materials and methods IL-1β and acupuncture was used to establish IDD model rats. $A_{2A}R$ agonist CGS-21680 and $A_{2A}R$ antagonist SCH442416 were used to investigate the therapeutical effects for IDD. Histological examination, western blotting analysis and RT-PCR were employed to evaluate the the association between $A_{2A}R$ and cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway.

Results $A_{2A}R$ activity of the intervertebral disc tissues was up-regulated in feedback way, and cAMP, PKA and CREB expression were also increased. But in general, IL-1β-induced IDD promoted the significant up-regulation the expression of inflammatory factors. The nucleus pulposus (NP) inflammation was exacerbated in result of MMP3 and Col-II decline through activating NF-κB signaling pathway. $A_{2A}R$ agonist CGS-21680 exhibited a disc protective effect through significantly increasing $A_{2A}R$ activity, then further activated cAMP/PKA signaling pathway with attenuating the release of TNF-α and IL-6 via down-regulating NF-κB. In contrast, SCH442416 inhibited $A_{2A}R$ activation, consistent with lower expression levels of cAMP and PKA, further leading to the acceleration of IDD.

Conclusions The activation of $A_{2A}R$ can prevent inflammatory responses and mitigates degradation of IDD thus suggest a potential novel therapeutic strategy of IDD.

Keywords Intervertebral disc degeneration, Nucleus pulposus, $A_{2A}R$, CAMP/PKA, NF-κB signaling pathway, Inflammatory damage

Introduction

Low back pain, as one of the most prevalent clinical disorders of the musculoskeletal system, is characterized by a range of chronic and progressively debilitating symptoms, significantly impairing the quality of human life [1]. Degenerative spinal disease resulting from intervertebral disc degeneration (IDD) is one of the primary causative factors for low back pain [2]. IDD is characterized by the

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necrosis of endplate chondrocytes, the rupture of the annulus fibrosis (AF), and apoptosis of nucleus pulposus (NP) cells [3]. Recent studies indicate that inflammatory hyperactivation is a significant factor contributing to apoptosis in NP cells [4]. During the degeneration process, inflammatory cells, such as macrophages, neutrophils, and monocytes, release significant quantities of tumor necrosis factor-α (TNF-α) and interleukin (IL), which would infiltrate intervertebral disc lesions, resulting in alterations to the local micro-environment. Studies have demonstrated that IL-1β directly induces aberrant prostaglandin metabolism in intervertebral disc cartilage and mediates the degradation of matrix proteins, ultimately resulting in cartilage endplate calcification and NP apoptosis [5]. Simultaneously, TNF-α stimulates the production of the matrix metalloproteinases (MMPs) in NP cells. The increased expression of MMP-3 leads to Collagen-II (Col-II) degradation, inducing AF disruption and NP cell apoptosis, thereby exacerbating IDD [6]. Inflammation plays a central role throughout the entire pathological process. Therefore, the identification of new targets that mitigate inflammation is crucial for reducing NP cell apoptosis in IDD. It is important to elucidate the precise pathogenesis of IDD linked to inflammatory signaling pathways [4].

Adenosine, an endogenous signal transduction molecule, binds to adenosine receptors (ARs) on the cell membrane surface to modulate inflammation. ARs, belonging to the family of G protein-coupled receptors (GPCRs), are widely expressed in various tissues, including A_1R, $A_{2A}R$, $A_{2B}R$, and A_3R [7]. A_1R and A_3R downregulate cAMP expression through coupling to GiPCR, whereas $A_{2A}R$ and $A_{2B}R$ upregulate cAMP expression through coupling to GsPCR. Among them, A_1R and $A_{2A}R$ exhibit high affinity for adenosine, whereas $A_{2B}R$ and A_3R display low affinity. $A_{2A}R$ plays an important role in the regulation of inflammation in various diseases. Ohta et al. suggested $A_{2A}R$ is a critical part of the physiological negative feedback mechanism for limitation and termination of both tissue-specifc and systemic inflammatory responses, and demonstrated that A2AR attenuates Concanavalin A-induced liver injury by up-regulating cAMP expression and reducing the secretion of TNF-α, IL-12, and IFN-γ [8]. This suggests that $A_{2A}R$ plays a distinct role in modulating inflammation and tissue damage in vivo. Wu et al. founded that $A_{2A}R$ expression in spinal nerve ligation rats is up-regulated after administration of an $A_{2A}R$ agonist [9], and activated $A_{2A}R$ is coupled to membrane stimulatory GsPCR to activate the downstream signaling pathway, inhibiting the phosphorylation of nuclear factor kappa-B (NF-κB), and thereby reducing inflammatory infiltration damage [10]. In addition, activated $A_{2A}R$ also improves neuronal plasticity and reduces neuropathic pain in rats in model of spinal nerve ligation [9]. Above reports demonstrate that $A_{2A}R$ plays a critical role in relieving pain and attenuating inflammatory environment. Nevertheless, the roles of $A_{2A}R$ in IDD haven't been studied, and the relationship between $A_{2A}R$ and IDD process is also unclear.

Activation of $A_{2A}R$ induces adenylyl cyclase activation, subsequently leading to the production of cyclic adenosine monophosphate (cAMP) [11]. cAMP, a second messenger, subsequently activates cAMP-dependent protein kinase A (PKA), which catalyzes AMP-response element protein (CREB) [9], thereby regulating target gene expression. Intrathecal injection of $A_{2A}R$ agonist resulted in a decrease in TNF-α expression in rat glial cells in vitro [12]. These findings suggest that $A_{2A}R$ might serve as a crucial target for attenuating inflammation in IDD by modulating the NF-κB pathway. Therefore, we hypothesized that the administration of the $A_{2A}R$ agonist CGS-21680 could inhibit the synthesis and secretion of TNF-α, IL-6, and MMP-3 in IDD rats by modulating the PKA/NF-κB signaling pathway, leading to a reduction in the inflammatory infiltration of endplate chondrocytes and NP cells. Furthermore, we employed the $A_{2A}R$ antagonist SCH442416 to inhibit $A_{2A}R$ activation and disrupt cAMP/PKA signal transduction. Subsequently, we assessed the impact of NF-κB activation on inflammatory infiltration in IDD.

To elucidate the role of A2AR in vivo, we established the IDD model following the previously described method by Kim and Yu et al., which is involved with lumbar disc puncture and intradiscal injection of IL-1β [5] to result in the inflammation and NP cells apoptosis. This study aimed to investigate the alterations of $A_{2A}R$ activity and its downstream molecular components in IDD, elucidate the potential mechanisms of $A_{2A}R$ regulating inflammatory responses, and then confirm the protective effects of CGS-21680 in decelerate IDD progression. The findings of this study may offer a new insight and therapeutic strategies for impeding IDD progression.

Materials and methods

Animals

A total of 32 male Sprague–Dawley (SD) rats (8 weeks, 200–220 g) were obtained from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All experimental procedures were complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication), and which were approved by the laboratory Animal Welfare & Ethics Committee at Bestcell Model Biological Center (BSMS 2023–09-27A). All animals were in the same specific pathogen-free (SPF) standard environmental conditions

(25 °C, light/dark cycle for 12 h) with free access to food, and allowed to move freely. The rats were randomly divided into four groups: the sham group (n = 8, S group), the IDD model group (n = 8, M group), the $A_{2A}R$ agonist group (n = 8, CGS-21680 group) and the $A_{2A}R$ antagonist group (n = 8, SCH442416 group).

Puncture and injection of drugs

The IDD model was established as previously described by Kim and Yu et al [5]. Briefly, rats were anesthetized with 0.3% sodium pentobarbital, and the vertebral bodies of L5-6 were confirmed by palpation. Next, a longitudinal incision was performed on the back skin of all rats and the surgical field of L5-6 disc was exposed to puncture with a 30-G needle (parallel to the endplate). First, the rats in S group received 25 μl saline injection into the NP center of L5-6 disc. The rats in M group, CGS group and SCH group all received 25 μl (4000 ng/ml, 400-01B, PeproTech, USA) IL-1β injection to establish IDD model as described. Then, rats in CGS group received 50 μl (0.1 mg/kg, HY-13201A, Med Chem Express, Beijing, China) CGS-21680 injection [13], and the rats in SCH group also received 50 μl (0.35 mg/kg, HY-103169, Med Chem Express, Beijing, China) SCH442416 injection [14]. Meanwhile, S group and M group were given the same amount (50 μl) of saline injection. Then the needle was kept still for about 30 s. The injection time was defined as the starting time (0 week).

Functional assessment

The gross locomotor function of IDD rats was scored according to the 21-point Bresnahan (BBB) locomotor rating scale that ranks complete paralysis as 0 points and normal locomotion as 21 points [15]. Put rats into an open basin, tap basin wall to make them move, and observe the movement of rats. Lifting, whipping, licking, or running of the paw during stimulation would be determined as positive avoidance response. The behavior tests were performed in the morning between 09:00 and 12:00 of the day of injection and 1, 4, and 8 weeks afterwards. All scoring tests were done in an open field by two observers blinded to the treatment.

Samples collection and biochemical analysis

After 8 weeks, all mice were sacrificed by receiving intraperitoneal injection of 10% sodium pentobarbital. The intervertebral disc tissue and blood samples were obtained. The lumbar spine containing L5-6 disk were collected and washed with PBS buffer for three times, then fixed overnight with 4% PFA. After decalcified in 10% EDTA solution for 4 weeks, tissue samples were embedded into paraffin blocks and sectioned into 4 μm thicknesses.

The concentration of cAMP, TNF-α and IL-6 in the intervertebral disc tissues and blood were measured using ELISA kits (ELK8207, ELK1396, ELK1158, ELK Biotechnology, Wuhan, China) according to the manufacturer instructions.

Histological examination

Hematoxylin–eosin (H&E) staining (AS1055B, Aspen, USA) and Safranin-O/Fast Green staining (G1053, Servicebio, Wuhan, China) were performed to analyze pathology according to the manufacturer instructions on the L5-6 disk site to inspect the damage of the punctured disk in each group. The grade of damage in intervertebral disks was evaluated according to the histological grading scale based on the cellularity and morphology of intervertebral discs [16].

Immunohistochemical and immunofluorescence staining were made on intervertebral disc tissue sections for analyzing expression with following antibodies: $A_{2A}R$ (1:100, Proteintech, Wuhan, China), PKA (1:200, abcam, Cambridge, Massachusetts), MMP3 (1:200, abcam, Cambridge, Massachusetts, USA), Collagen II (1:200, Proteintech, Wuhan, China). The immunoreactivity was quantified using commercial software (Image Pro Plus, Media Cybernetics, Inc., Rockville, Maryland, USA) to assess the percent of positive area in the pictures.

Western blotting analysis

Total protein was extracted from NP tissues with radio immunoprecipitation assay (RIPA) buffer containing phosphatase and protease inhibitors. Then, bicinchoninic acid (BCA) protein assay kit (AS1086, Aspen, USA) was used to determine the protein concentrations. Thirty micrograms of total protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then electro-transferred to the transmembrane PVDF (Millipore, Billerica, MA, USA), blocked with 5% milk protein for 1 h. After that, the target membranes were incubated with following primary antibodies overnight at 4 °C: anti-$A_{2A}R$ (1:500, Proteintech, Wuhan, China), anti-PKA(1:1000, abcam, Cambridge, Massachusetts), anti-CREB (1:1000,Proteintech,Wuhan,China), NF-κB (1:1000, Gene Tex, China), anti-MMP3(1:1000, abcam, Cambridge, Massachusetts, USA), anti-IL-6(1:1–000, Gene Tex, China), anti-TNF-α (1:1000, Gene Tex, China) and anti-Collagen II (1:500, Proteintech, Wuhan, China) polyclonal antibodies and β-actin (1:2000, Antgene, Wuhan, China). The membrane was immunoblotted with the corresponding secondary antibody (Goat-anti-rabbit, Goat-anti-mouse, KPL,

USA). The chemiluminescence method was performed to obtain the immunoreactive bands. The images were captured and semi-quantitatively analyzed by Quantity One (Bio-Rad, USA).

Measurement of pro-Inflammatory cytokines mRNA levels by RT-PCR

Total RNA was extracted from the L5-6 disk tissues using RNA Tissue Mini Kit (Novoprotein, Shanghai, China) according to the manufacturer instructions. Reverse transcription and cDNA synthesis were accomplished using Prime Script RT Master Mix Perfect Real Time (Takara, RR037A, Kusatsu, Shiga, Japan). Real-time polymerase chain reaction was performed to detect the expression of various cytokines by Quanti Fast SYBR Green PCR Kit (Qiagen, 204,057, Hilden, Germany) and Bio-Rad CFX96 (Bio-Rad, Hercules, CA, USA) according to the manufacturer instructions. The PCR processes were as follows: 94 °C for 3 min and 45 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s. Then, PCR was terminated by incubation at 72 °C for 5 min. PCR primers were shown in Table 1. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

All statistic data were expressed as mean ± SD, and firstly evaluated for normal distribution using Shapiro–Wilk test. Then, the results were compared by one-way ANOVA followed with Tukey's or Games-Howell's post hoc test. The Kruskal-Wallisnon-parametric test was also used to evaluate non-normal distribution variables. The mortality rate in four groups were compared by log rank test of Kaplan–Meier curves. The value of $P<0.05$ was considered statistically significant. The Graph Pad Prism 5.0 software (San Diego, CA, USA) was employed to perform the statistical analysis.

Table 1 PCR primers

	Sequence (5′–3′)
GAPDH	F: 5′-CGCTAACATCAAATGGGGTG -3′ R: 5′-TTGCTGACAATCTTGAGGGAG -3′
A2AR	F: 5′-GCTGACATTGCAGTGGGTGT -3′ R: 5′-CTAAAGATGGAACTCTGCGTGAG -3′
PKA	F: 5′-GATTGGGAGGTTCAGTGAGCC-3′ R: 5′-GTCCACAGCTTTGTTGTAGCCTT-3′
CREB	F: 5′-TGACTTATCTTCTGATGCACCAG-3′ R: 5′-GCATTGGTCATGGTTAATGTCT-3′
IL-6	F: 5′-GGTTTGGAGAATCTATGAATGGTGG-3′ R: 5′-GCTGGAAAGAAGTCTGAGGAAGG-3′
TNF-α	F: 5′-CAGGCGGTGCCTATGTCTC-3′ R: 5′-CGATCACCCCGAAGTTCAGTAG-3′
NF-κB	F: 5′ -CAACCTGTGCCTACACTTCAAC-3′ R: 5′-CGTCTGGCAGCTTGATGGT-3′
NLRP3	F: 5′-AACCAGAGCCTCACTGAACTGG-3′ R: 5′-AGAGCAGATGCTTCAGTCCCAC-3′

F, forward; R, reverse

Results

Experimental procedure and survival

Schematic diagram of the experimental procedure was performed according to Fig. 1A. As depicted in Fig. 1B, all rats in the S group survived until the end of the study, along with 7 rats in the M group, 7 rats in the CGS-21680 group, and 4 rats in the SCH442416 group. In the M group, one rat died on the 3rd day after the successful model establishment, while in the CGS-21680 group, one rat died on the 7th day. In the SCH442416 group, four rats died on the 4th, 6th, 7th, and 9th days, respectively. The survival rate of CGS-21680 group was higher than SCH442416 group ($P>0.05$).

Histological staining and grading scores

We assessed the histological structure of the L5-6 intervertebral disc using HE staining and Safranin-O/Fast Green staining (Fig. 1C). Additionally, we presented the histological grading scores for the various groups (Fig. 1D).

The morphological structure of the disk in S group was remained intact and regular, and the corresponding histological grading score was about 5. In contrast, the concentric circle structures of AF were disrupted in both the M group and the SCH442416 group, and fewer chondrocytes were observed in the NP tissue compared to the S group. This observation indicates structural damage at grade II or III, with a histological grading score of approximately 10. The degenerated disc sites were additionally characterized by the presence of fibrocartilage tissue, scar tissue, and various morphological cells. However, the aggravation of fibrosis in NP significantly decreased and NP cells increased after CGS-21680 injection. In CGS-21680 group, the structure destruction in grade I or II was much milder and the histological grading score was higher than that in the M group and SCH442416 group ($P<0.05$).

Locomotor function assessment with BBB score

The locomotor function of IDD rats was assessed by BBB score which was shown in Fig. 1E. One week later, the BBB scores in all groups showed a significant decrease. Nevertheless, the BBB scores of the M group and the SCH442416 group were significantly much lower than those of the S group ($P<0.05$). In the CGS-21680 group, the average BBB score was 16 points, representing an approximately 5-point increase compared to both the M group and the SCH442416 group ($P<0.05$). After four weeks, the BBB scores in all groups increased. The

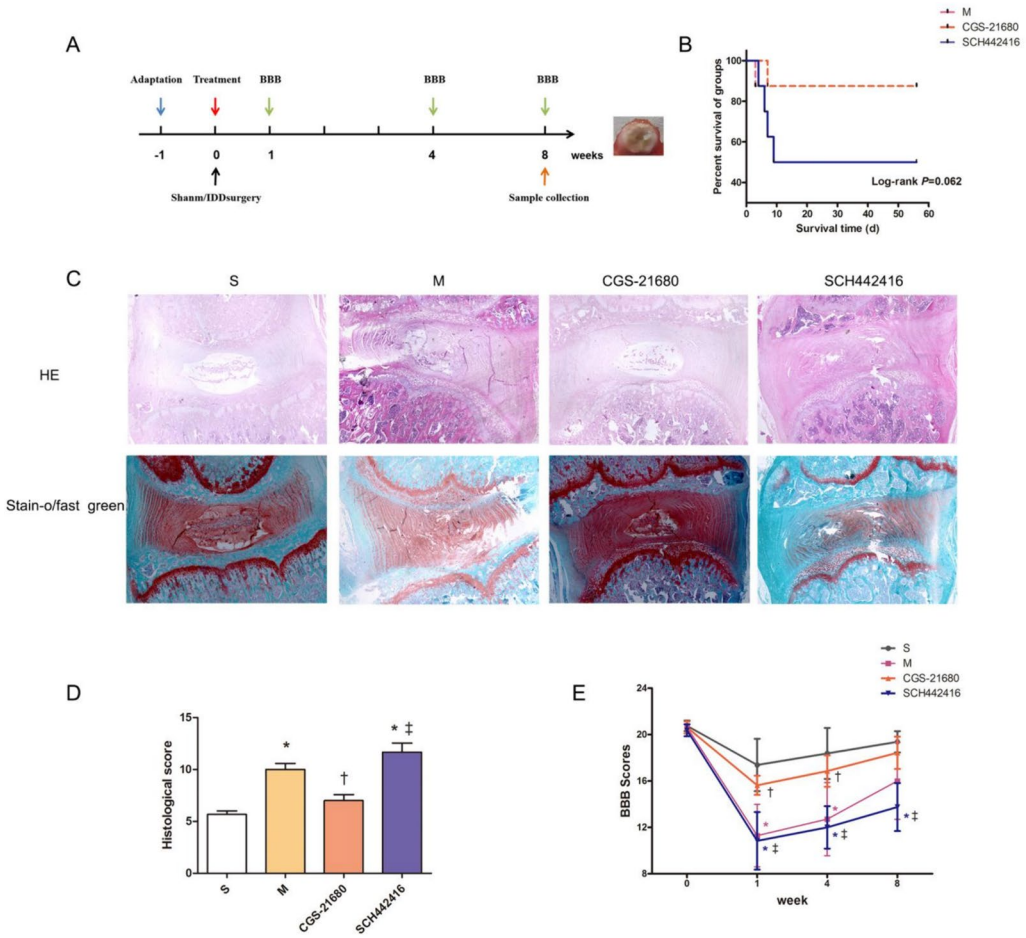

Fig. 1 Schedule of experiment and scoring. **A** Schematic diagram of the experimental procedure. **B** Survival curve. **C** HE staining and Safranin O-fast green staining in each group. **D** Histological score changes in the L5-6 intervertebral disk. Data represented mean ± SD (n = 4). **E** BBB scores. Data represented mean ± SD.*$P < 0.05$ versus S group, $^{\dagger}P < 0.05$ versus M group, $^{\ddagger}P < 0.05$ versus CGS-21680 group. S, sham operation group; M, IL-1β-induced IDD model group; CGS-21680, $A_{2A}R$ agonist CGS-21680 group; SCH442416, $A_{2A}R$ antagonist SCH442416 group

average scores in the M group and the SCH442416 group were approximately 12 and 13, respectively, significantly lower than those in the S group ($P < 0.05$). In the CGS-21680 group, the average score was 17 points, which was significantly higher than that in both the M group and the SCH442416 group ($P < 0.05$). After eight weeks, the BBB scores continued to increase, but there was no significant difference between the S group and the M group. The BBB scores in the CGS-21680 group still remained higher than that in the SCH442416 group ($P < 0.05$).

$A_{2A}R$ level changes in IDD model and CGS-21680 activated the expression of $A_{2A}R$ activation

$A_{2A}R$ expression was assessed through immunofluorescence staining, RT-PCR, and Western blotting, as illustrated in Fig. 2A–C. Positive $A_{2A}R$ expression in intervertebral disc tissues, as revealed by immunofluorescence staining, indicated with yellow arrows in drawing of partial enlargement. Furthermore, a significant upregulation in $A_{2A}R$ mRNA and protein levels was detected through RT-PCR and Western blotting (Fig. 2B, C). In the CGS-21680 group, $A_{2A}R$ mRNA expression was significantly higher than in the M group ($P < 0.05$), and similar trends were observed for $A_{2A}R$ protein levels. Compared with the M group and CGS-21680 group, the SCH442416 group appeared to a great decrease in the expression levels of $A_{2A}R$ with significant difference ($P < 0.05$).

The effects of $A_{2A}R$ on the downstream PKA, CREB and NF-κB pathways

The cAMP level was measured using ELISA. There was no significant difference in cAMP expression in plasma among the four groups, as shown in Fig. 3A. However, the cAMP levels in intervertebral disc tissues, as illustrated in Fig. 3B, showed a slight increase in the M group compared to the S group. Meanwhile, the downstream

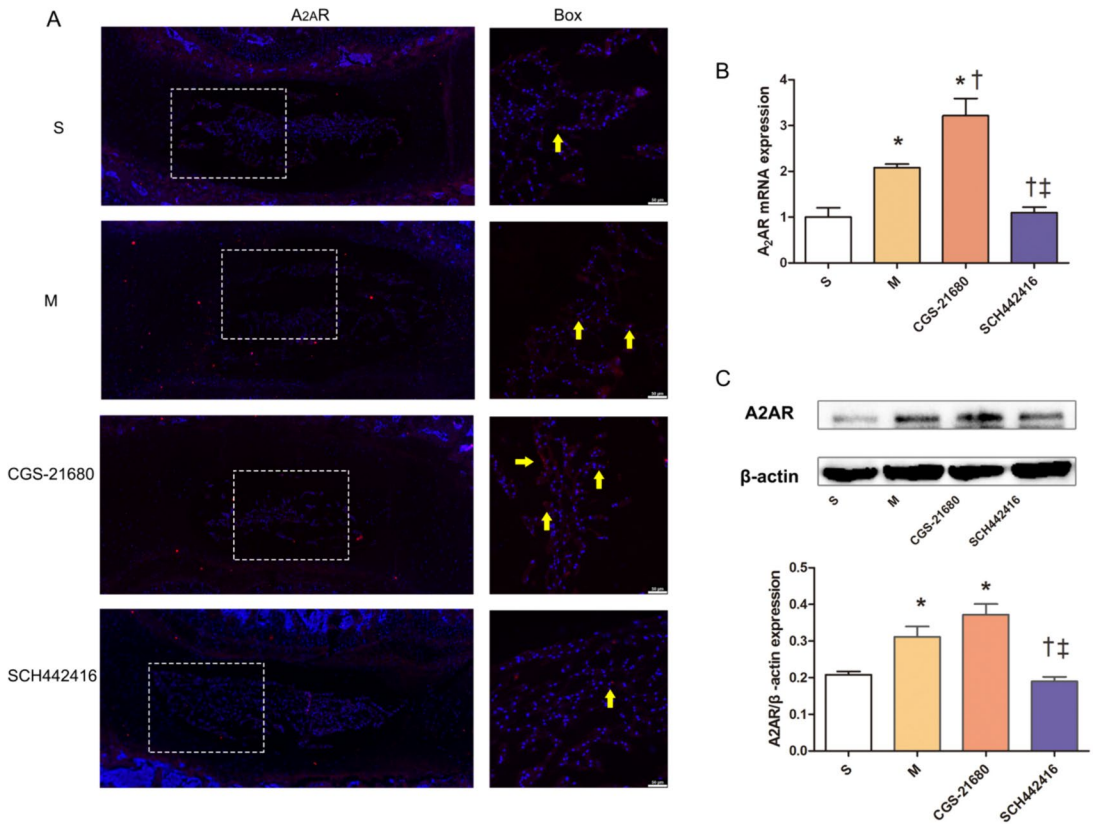

Fig. 2 $A_{2A}R$ expression in rat IDD model. **A** Immunofluorescence staining images of $A_{2A}R$ of NP cells and the drawing of partial enlargement in box (positive expression indicated with the yellow arrows). **B** RT-PCR and **C** Western blot were used to detect the expression level of $A_{2A}R$ in four groups. Data represented mean ± SD. Scale bars = 50 μm. *$P < 0.05$ versus S group, †$P < 0.05$ versus M group, ‡$P < 0.05$ versus CGS-21680 group. S, sham operation group; M, IL-1β-induced IDD model group; CGS-21680, $A_{2A}R$ agonist CGS-21680 group; SCH442416, $A_{2A}R$ antagonist SCH442416 group

expression of PKA and CREB, as depicted in Fig. 3C–H, was slightly higher than that in the S group. In contrast, the cAMP levels in the CGS-21680 group were significantly upregulated ($P < 0.05$), and there was also a notable increase in PKA mRNA expression, as shown in Fig. 3C ($P < 0.05$). Meanwhile, more PKA positive expression (Fig. 3D**,** indicated with the yellow arrows in drawing of partial enlargement) was also observed in CGS-21680 group. The mRNA levels of CREB in the CGS-21680 group were significantly higher than those in both the S group and the M group ($P < 0.05$), as depicted in Fig. 3H. Administering SCH442416 resulted in a significant reduction in the expression of the aforementioned substances ($P < 0.05$). Western blotting results revealed that CGS-21680 further elevated the protein levels of PKA and CREB compared to the S group ($P < 0.05$), and these levels were subsequently reduced following SCH442416 treatment, as illustrated in Fig. 3E–G.

Pathological activation of the NF-κB signaling pathway has been closely associated with inflammation in IDD. We also investigated the impact of $A_{2A}R$ on NF-κB alterations, as shown in Fig. 3E, I, and J. In our study, Western blotting and RT-PCR results demonstrated that NF-κB expression was increased in the M group compared to the S group, which was obviously decreased in the CGS-21680 group ($P < 0.05$). However, NF-κB expression was significantly upregulated in the SCH442416 group ($P < 0.05$), suggesting the activation of the NF-κB signaling pathway. The NLPR3 mRNA levels were significantly decreased in the CGS-21680 group compared to both the M group and the SCH442416 group (Fig. 3K, $P < 0.05$).

Activation of $A_{2A}R$ reduce inflammatory response

To investigate inflammatory changes in IDD, the contents of TNF-α and IL-6 was analyzed by ELISA, RT-PCR and Western blotting. As shown in Fig. 4A, B, TNF-α levels in the plasma and intervertebral disc tissues were elevated in the M group and the SCH442416 group compared to the S group, respectively. The difference was more significant in the intervertebral disc tissues. The CGS-21680 group exhibited lower TNF-α expression levels ($P < 0.05$). According to the results of RT-PCR (Fig. 4C) and Western blotting (Fig. 4G, H), TNF-α expression in the intervertebral disc tissues of the M group and the

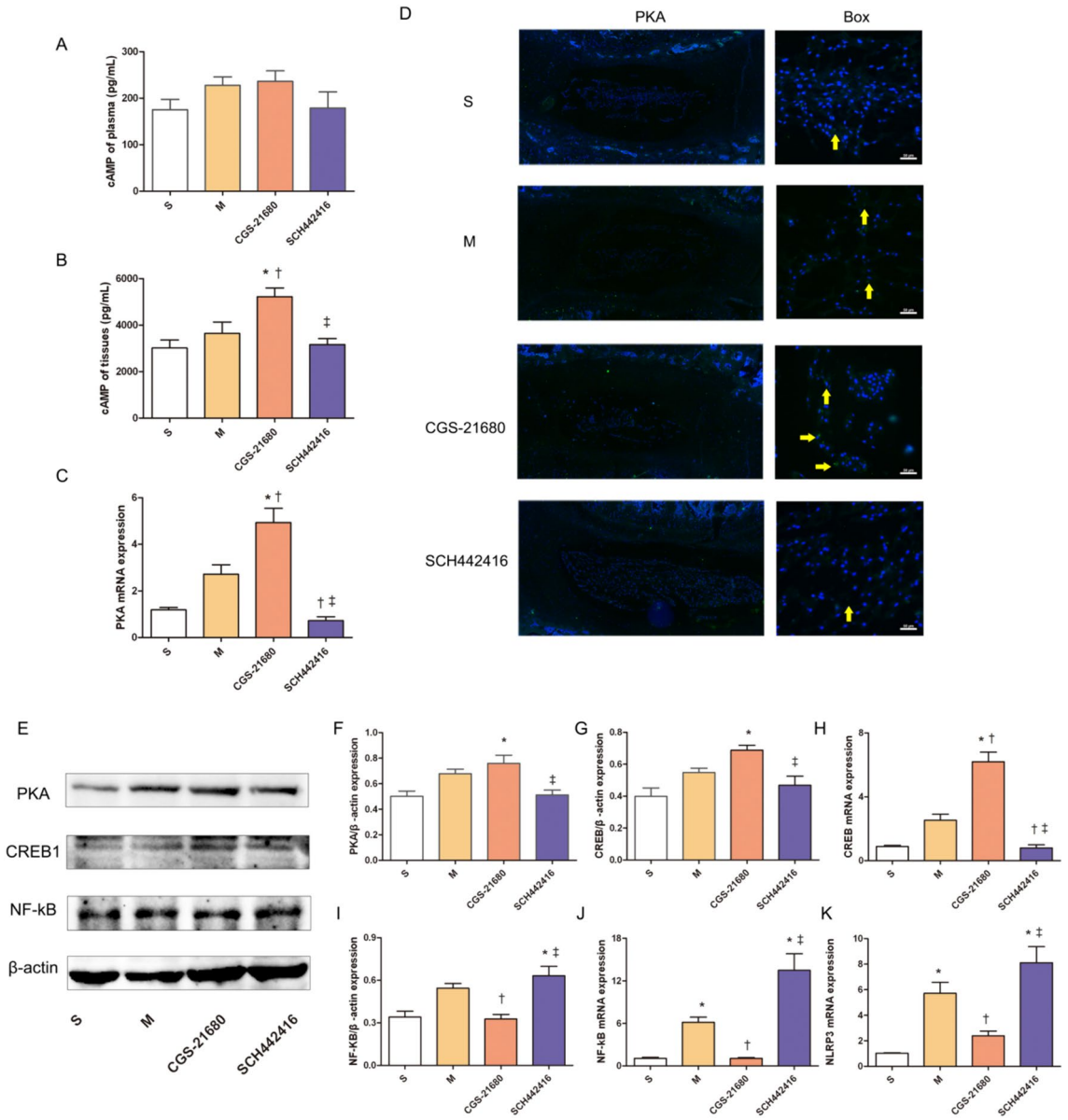

Fig. 3 Effects of $A_{2A}R$ on the PKA/NF-κB pathway. **A**, **B** cAMP level was detected in the plasma and intervertebral disc tissues by ELISA. **C** RT-PCR results of PKA mRNA. **D** Images of PKA in each group with immunofluorescence staining in four groups and the drawing of partial enlargement in box (positive expression indicated with the yellow arrows). **E**–**I** Western-blot results of PKA, CREB and NF-κB in four groups. (**H**, **J**, **K**) RT-PCR results of CREB, NF-κB and NLRP3 mRNA. Scale bars = 50 μm. Data represented mean ± SD. Scale bars = 200 μm.*$P < 0.05$ versus S group, $^{\dagger}P < 0.05$ versus M group, $^{\ddagger}P < 0.05$ versus CGS-21680 group. S, sham operation group; M, IL-1β-induced IDD model group; CGS-21680, $A_{2A}R$ agonist CGS-21680 group; SCH442416, $A_{2A}R$ antagonist SCH442416 group

SCH442416 group was significantly higher than that in the S group ($P < 0.05$). Conversely, TNF-α mRNA and protein levels were markedly reduced following CGS-21680 injection ($P < 0.05$).

The IL-6 levels of M group and SCH442416 group in both the plasma (Fig. 4D) and intervertebral disc tissues (Fig. 4E) were significantly higher than S group ($P < 0.05$).The levels of IL-6 mRNA and protein were notably increased in both the M group and the SCH442416 group in comparison to the S group (Fig. 4F, G, I, P < 0.05). After the administration of the $A_{2A}R$ agonist CGS-21680, the expression of IL-6 mRNA and protein levels was significantly reduced ($P < 0.05$).

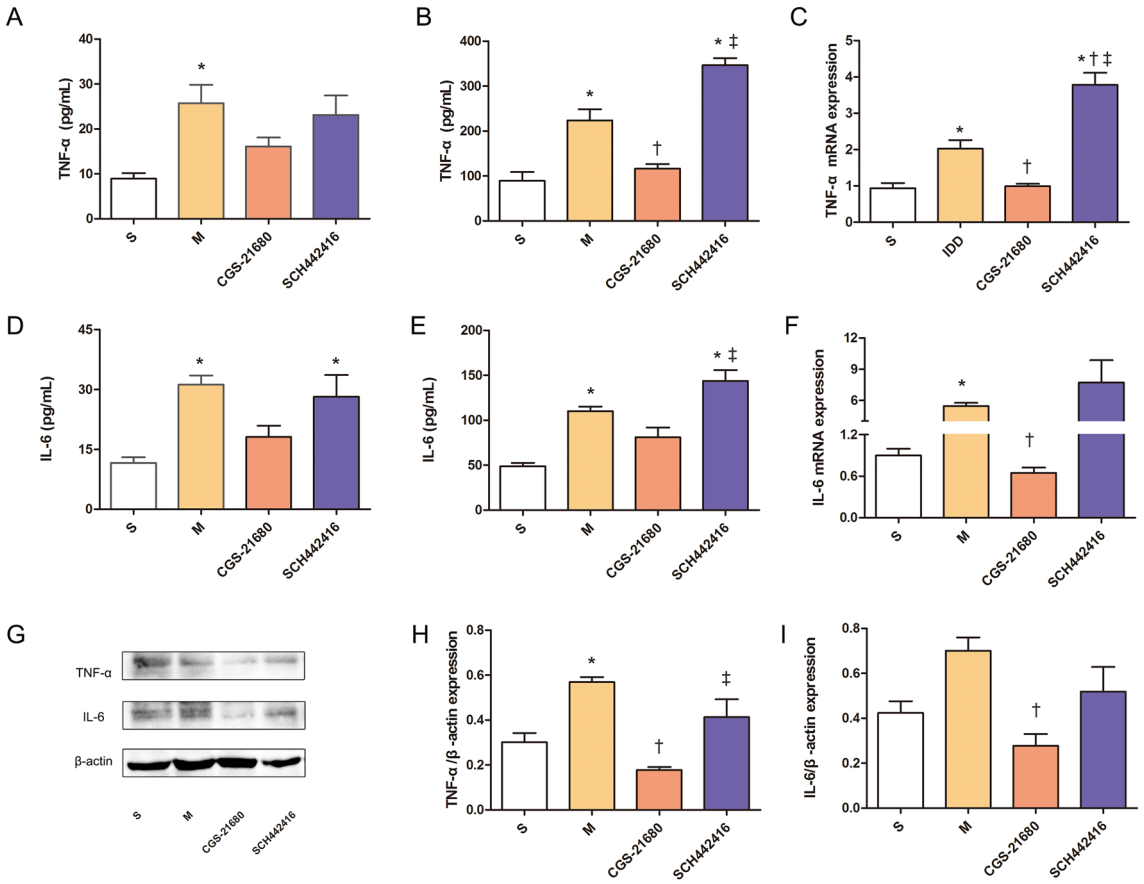

Fig. 4 Effects of $A_{2A}R$ on the inflammatory cytokines. **A**, **B** TNF-α and **D**, **E** IL-6 expression in the plasma and intervertebral disc tissues by ELISA. **C**, **F** RT-PCR results of TNF-α and IL-6 mRNA level. **G**–**I** Western-blot results of TNF-α and IL-6 protein in four groups. Data represented mean ± SD. Scale. *$P < 0.05$ versus S group, †$P < 0.05$ versus M group, ‡$P < 0.05$ versus CGS-21680 group. S, sham operation group; M, IL-1β-induced IDD model group; CGS-21680, $A_{2A}R$ agonist CGS-21680 group; SCH442416, $A_{2A}R$ antagonist SCH442416 group

Effects of $A_{2A}R$ activation on the expression of MMP3 and Col-II

The degradation of the extracellular matrix is a primary factor contributing to the pathogenesis of IDD. Reduced levels of Col-II and increased MMP3 within the NP tissue have been identified as significant contributors to NP cell apoptosis and extracellular matrix degradation. Immunofluorescence staining revealed the increase of MMP3 positive expression in the M group (Fig. 5A), and SCH442416 treatment also led to a substantial elevation in MMP3 expression. Consistent with these results, it was observed that the levels of Col-II were markedly reduced in both the M group and the SCH442416 group compared to the S group ($P < 0.05$). The CGS-21680 group exhibited a significant decrease in MMP3 levels (Fig. 5B) and a significant increase in Col-II expression (Fig. 5B–D). Taken together, this data suggested that the up-regulation of $A_{2A}R$ alleviated extracellular matrix and NP cell degradation.

Discussion

In this study, we revealed the important role of $A_{2A}R$ in the IDD process. The major findings were summarized as follows: (1) The $A_{2A}R$ activity of the intervertebral disc tissues was up-regulated in feedback way when the elevated IL-1β induced inflammatory disc degeneration; (2) Although the downstream cAMP/PKA and CREB expressions were slightly increased, the IL-1β-caused IDD still strongly up-regulated NF-κB signaling pathway to up-regulate inflammatory cytokines and exacerbate the NP inflammation; and (3) CGS-21680 could significantly activate $A_{2A}R$ activity, up-regulate downstream cAMP/PKA, and subsequently alleviate inflammation by down-regulating the NF-κB signaling pathway. To the best of our knowledge, this is the first study to focus on the role of A2AR and the related mechanism of CGS-21680 in reducing inflammatory damage. Overall, $A_{2A}R$ might be a promising novel therapeutic target for the treatment of IDD.

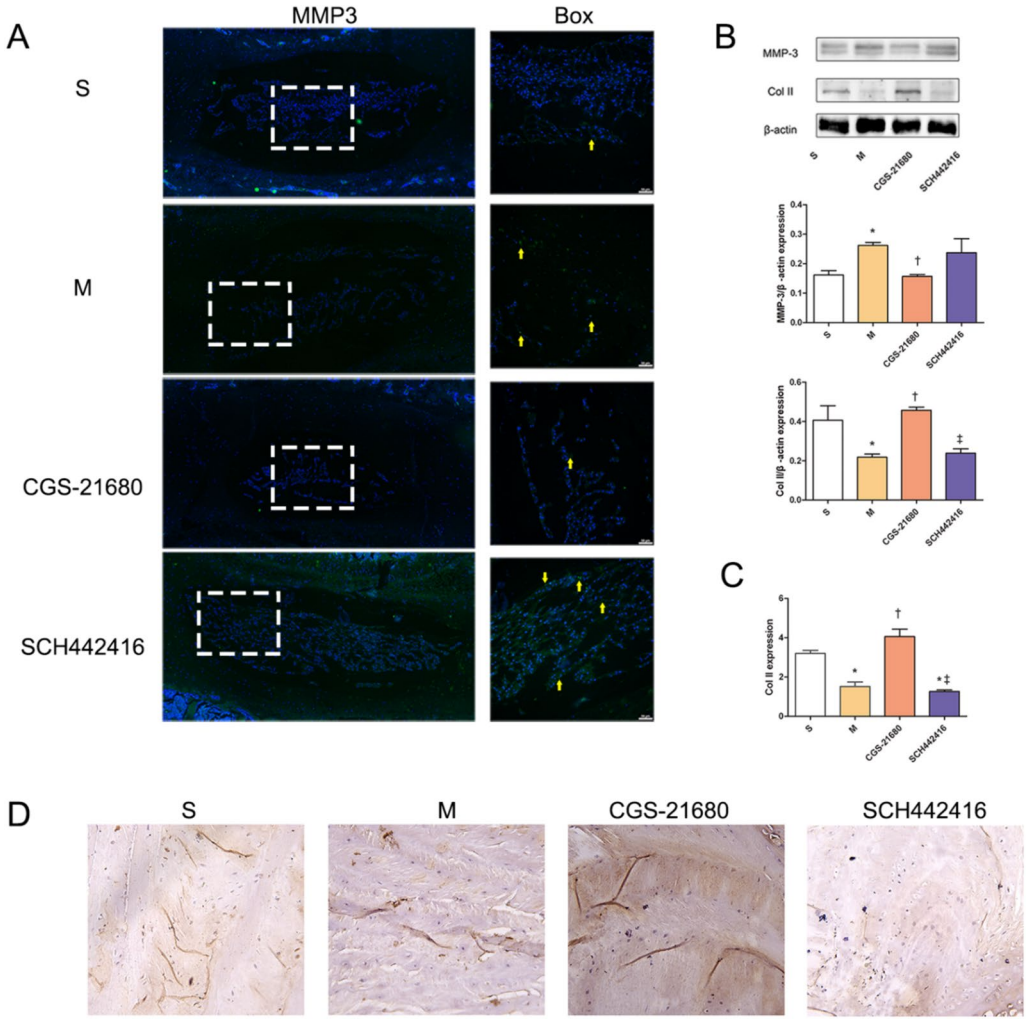

Fig. 5 Effect of $A_{2A}R$ up-regulation on the expression of matrix metalloproteinase and type II collagen. **A** images of MMP3 in each group with immunofluorescence staining and and the drawing of partial enlargement in box (positive expression indicated with the yellow arrows) (Scale bars = 50 μm). **B** MMP3 and type II collagen levels were detected by western-blot. **C**, **D** Immunohistochemistry results of type II collagen in each group (Scale bars = 50 μm). Data represented mean ± SD.*$P < 0.05$ versus S group, $^{\dagger}P < 0.05$ versus M group, $^{\ddagger}P < 0.05$ versus CGS-21680 group. S, sham operation group; M, IL-1β-induced IDD model group; CGS-21680, $A_{2A}R$ agonist CGS-21680 group; SCH442416, $A_{2A}R$ antagonist SCH442416 group

IDD is a complex biological process involving a chronic inflammatory response. Inflammatory mediators, such as TNF-α, IL-1β, and IL-6, have been demonstrated to be crucial pro-inflammatory cytokines that directly accelerate the degeneration of NP tissue [17]. In this study, we found that IL-1β led to a deteriorated histological structure, a decrease in histological grading scores, and a reduction in locomotor activity (as shown in Fig. 1C). Consistent with a previous study, IL-1β exacerbated the severity of disc degeneration and inflammation [5].

Currently, the treatment of IDD mainly revolves around physiotherapeutic and anti-inflammatory therapies [18, 19]. However, these conservative treatments have not been able to completely reverse or alleviate the progression of IDD. Meanwhile, surgical interventions still carry certain intraoperative risks and postoperative complications. Therefore, it is important to discover new therapeutic targets and develop safe and effective treatment strategies. Previous researches have documented that $A_{2A}R$ plays a significant role in various diseases, including lung injury, myocardial ischemia, and spinal diseases [9, 20, 21]. Both $A_{2A}R$ and the downstream cAMP/PKA pathway are involved in the inflammatory response [20]. Wu et al. also reported that up-regulation of $A_{2A}R$, cAMP and PKA expression could alleviate the pain and tissue damage of spinal cord site in rats with spinal nerve

ligation [9]. However, the exact effects of $A_{2A}R$ on the inflammatory response and NP cells apoptosis in IDD were not fully understood.

The changes of $A_{2A}R$ activity and its downstream signaling pathways

Previous studies have reported that $A_{2A}R$, as a feedback inhibitor of inflammation, is up-regulated in response to agents that activate NF-κB during the inflammatory process [22, 23]. Additionally, TNF-α or IL-1β can increase $A_{2A}R$ expression and enhance function in monocytes or peripheral white blood cells by preventing receptor desensitization [23]. Wu and Castro also reported that $A_{2A}R$ expression and activity were up-regulated in spinal nerve ligation and osteoarthritis (OA) chondrocytes, respectively [9, 24]. Consistent with above reports, our results demonstrated that the expressions of $A_{2A}R$ in NP tissue were up-regulated in response to IL-1β intervention. Given that the signaling of $A_{2A}R$ involves a sequential process where it couples with $G_S\alpha$ to stimulate the downstream cAMP/PKA and CREB pathways [11, 25, 26], we proceeded to measure and observed that IL-1β-induced IDD resulted in a slight increase in the expression of cAMP, PKA, and CREB in intervertebral disc tissues compared to the S group. Meanwhile, it led to a significant increase in the amount of the transcription factor NF-κB in the M group compared to the S group (Fig. 3). These results indicated that the activation of NF-κB, induced by IL-1β, played a predominant role in IDD rather than the cAMP/PKA and CREB signaling pathways mediated by up-regulated $A_{2A}R$.

NF-κB plays important role in a multitude of pathophysiological processes, including inflammation, cell apoptosis, and stress responses [27, 28]. It is also associated with the release of inflammatory cytokines that play a role in IDD. Recent published evidence has revealed that NF-κB activation promotes the over-expression of pro-inflammatory cytokines and matrix-degrading enzymes, exacerbating IDD [29]. Consistent with previous reports [30], the TNF-α and IL-6 contents, as well as mRNA and protein expressions in the intervertebral disc tissues of the M group, were significantly increased compared to the S group (Fig. 4). These findings further demonstrated that NP inflammation was exacerbated through the up-regulation of the NF-κB activation pathway. The protein expression of MMPs and Col-II reflects NP cell anabolism. The mature NP is rich in proteoglycans and Col-II, and the increased expression of MMP3 accelerates the degradation of the matrix, including proteoglycan and Col-II [31, 32]. Consistent with previous publications [31, 32], our results demonstrate a significant increase in MMP3 protein expression in the M group due to the activation of pro-inflammatory factors. This exacerbated the degradation of the extracellular matrix, including Col-II, and consequently impairs NP function.

The protective effect of $A_{2A}R$ agonist CGS-21680 on $A_{2A}R$ and their downstream signaling pathways in IDD

Previous reports have indicated that CGS-21680, an $A_{2A}R$-specific agonist, exhibits potent and long-lasting anti-inflammatory effects [9, 12, 33]. It can suppress pro-inflammatory cytokines and alleviate neuropathic pain in peripheral immune cells and nerve injury models. Loram and colleagues assessed the impact of $A_{2A}R$ agonist on neuropathic pain using a chronic constriction injury model [12, 33]. They discovered that a single intrathecal bolus injection of $A_{2A}R$ agonist reversed neuropathic pain by activating the PKA pathway and increasing the anti-inflammatory cytokine IL-10. This approach could potentially offer a novel therapeutic method for treating neuropathic pain. Fozard et al. administered CGS-21680 (0.1 mg/kg) intratracheally to suppress allergic airway inflammation [13]. Their demonstration indicated that local administration of CGS-21680 at a dose of 0.1 mg/kg was beneficial for treating asthma in a rat model of allergic asthma. Therefore, we evaluated the therapeutic effect of a single local administration of CGS-21680 (0.1 mg/kg) in our study. We also employed SCH442416 (0.35 mg/kg) to elucidate the potential mechanism of $A_{2A}R$ in IDD [14]. CGS-21680 significantly mitigated the structural damage associated with IDD, resulting in a normal morphology and lower histological grading score compared to the M group (Fig. 1). Additionally, locomotor behavioral performance was notably improved. Conversely, SCH442416 exacerbated the structural damage and led to worse histological grading scores and locomotor BBB scores compared to the CGS-21680 group.

Previous studies have provided support that the expression and function of $A_{2A}R$ were both up-regulated by factors such as TNF-α and IL-1β [22, 23]. The expression and activity of $A_{2A}R$ were further enhanced following CGS-21680 stimulation (Fig. 2). Simultaneously, SCH442416 substantially inhibited the expression and function of $A_{2A}R$, consistent with previous research [9]. Researchers have demonstrated that $A_{2A}R$ agonist increase the expression of cAMP, PKA and CREB, whereas $A_{2A}R$ antagonist inhibit $A_{2A}R$/cAMP/PKA pathway in neuropathic pain [9, 34]. As anticipated, the mRNA and protein levels of cAMP and PKA were significantly up-regulated in the CGS-21680 group, with a greater magnitude of increase compared to the M group (Fig. 3). Transcriptional activation of CREB is critically dependent on PKA [26]. Therefore, treatment with CGS-21680 induced CREB activation, which further downregulated inflammation. In line with previous descriptions, the selective $A_{2A}R$ antagonist SCH442416

clearly exhibited an inhibitory effect on the activation of the PKA pathway, resulting in a significant decrease in cAMP, PKA, and CREB expression [34, 35]. The above in vivo results suggested that the activation of the cAMP/PKA/CREB signaling pathway may explain the improvement in the degradation of NP tissues and locomotor function.

In order to further investigating the molecular mechanisms regulated by CGS-21680, we assessed the expression of NF-κB downstream. The PKA/CREB signaling pathway is well-known for its anti-inflammatory and anti-apoptotic effects due to its inhibition of the NF-κB pathway [26, 34]. The underlying molecular mechanism by which PKA/CREB activation results in NF-κB down-regulation in IDD remains unclear. As reported by Ali Masjedi, $A_{2A}R$ signaling suppresses the anti-tumor functions of T cells in mice through the PKA/CREB signaling pathway [35]. It also prevents the secretion of pro-inflammatory cytokines by inhibiting the activation and protein expression of NF-κB. Furthermore, Harbrecht et al. reported that the activation of cAMP/PKA under inflammatory conditions could inhibit the NF-κB signaling pathway and regulate the expression of pro-inflammatory genes, such as TNF-α and intercellular adhesion molecule-one [36]. Consistent with the above studies, our experimental results demonstrated that CGS-21680 activated $A_{2A}R$ signaling, and the up-regulation of PKA/CREB in the CGS-21680 group resulted in a significant down-regulation of NF-κB protein and gene expression. Conversely, SCH442416 inhibited $A_{2A}R$ expression and function, leading to the down-regulation of the PKA/CREB pathway, which resulted in the activation of NF-κB, as measured by RT-PCR and Western blotting (Fig. 3), in line with other research [35]. NLRP3, one of the most studied inflammasomes, consists of three components: the NOD-like receptor, cysteine protease caspase-1, and the adaptor protein apoptosis-associated speck-like protein containing a CARD [37, 38]. NLRP3 inflammasomes are activated by the NF-κB signaling pathway [39]. Our study found that mRNA expression of NLRP3 in the CGS-21680 group was significantly decreased, leading to the inhibition of caspase-1 activation. Consequently, the inflammation and programmed cell death process were blocked. These aforementioned results once again suggest the potential for $A_{2A}R$ to regulate the modulation of the NF-κB pathway and NLRP3.

Furthermore, we assessed the impact of $A_{2A}R$ on changes in inflammatory cytokines. Both the mRNA and protein expressions of TNF-α and IL-6 in NP tissues were significantly reduced in the CGS-21680 group compared to the M group (Fig. 4). This reduction can be attributed to the activation of the PKA/CREB pathway, which inhibits NF-κB. On the contrary, the down-regulation of PKA/CREB in the SCH442416 group led to the release of pro-inflammatory TNF-α and IL-6 as a result of NF-κB pathway activation. This, in turn, exacerbated extracellular matrix degradation and impaired NP function. In summary, the up-regulation of $A_{2A}R$ activated the PKA/CREB pathway to inhibit NF-κB, thereby mitigating the inflammatory response in IDD.

Previous studies have reported that inflammation is one of the primary contributing factors to NP degradation [31]. This is characterized by a reduction in the inherent collagen content and an increase in matrix-degrading enzymes [40]. Therefore, we assessed the expression of degeneration-associated factors, such as MMP3 and Col-II, which are related to IDD. In this study, CGS-21680 not only decreased the gene and protein levels of MMP3 but also enhanced Col-II expression, effectively reversing IDD. Thus, the activation of $A_{2A}R$ could be a promising therapeutic target for slowing down the progression of IDD.

Interestingly, caffeine is a natural $A_{2A}R$ antagonist, and its detailed mechanism involved in the progression of IDD remains unclear [41]. A clinical study from Poland including 609 healthcare workers found that excessive consumption of coffee (≥ 6 cups per day) significantly increased the likelihood of recurring low back pain (LBP) (OR = 16.69; 95% CI 8.77–43.36; $p < 0.001$) [42]. Meanwhile, a study conducted in China involving 969 participants found a positive correlation between coffee consumption and LBP risk [43]. Ahn also demonstrated a significant association between coffee intake and the occurrence of LBP [44]. However, IDD is the cause of LBP, above suggested caffeine might block $A_{2A}R$ to regulate IDD and induce the the occurrence of LBP. The further animal or cell experiment is needed to explore the effect and mechanism of drinking coffee on the exacerbation of IDD progression.

Study limitations

There were certain limitations to the present research. (1) The effect of CGS-21680 and SCH442416 on the immune cell subtypes in NP tissues is unclear, and it is important to to uncover the mechanism of treatment in this model. The response of different immune cell subtypes to treatments of CGS-21680 and SCH442416 is considered to evaluate in IDD model for the further study in the future; and (2) We highly acknowledge that the sample size is a weak point in our study which may affect the generalizability of the findings. Although the mortality of SCH442416 group was higher than other groups, the survival rate still presented no statistical difference. It is not really known that 50% of the animals died in SCH442416 group was due to the deleterious action or drug effect. A larger sample in future study will be made to investigate

the effect of SCH442416 on $A_{2a}R$ and further explore its function in the intervertebral disc.

Conclusions

In conclusion, our study showed that CGS-21680 could significantly up-regulate $A_{2A}R$ activity and activate the cAMP/PKA/CREB pathway to inhibit NF-κB signaling pathway, and then further alleviated the inflammation in IDD. Overall, activation of $A_{2A}R$ might be a potential novel therapeutic strategy for the improvement and prevention of IDD.

Abbreviations

IDD	Intervertebral disc degeneration
AF	Annulus fibrosis
NP	Nucleus pulposus
$A_{2A}R$	Adenosine A_{2A} receptor
cAMP	Cyclic adenosine monophosphate
PKA	Protein kinase A
TNF-α	Tumor necrosis factor-α
IL-6	Interleukin-6
MMP	Matrix metalloproteinase
AR	Adenosine receptor
NF-κB	Nuclear factor kappa-B

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Author contributions

Liu and Li took part in the designing of the experiments, contributed reagents, materials and analysis tools. Fang, Cai, Wang, Zhou and Zhou run the experiments. Liu, Li and Fang wrote the manuscript. Yao, Zhang and Wei participated in the analyzing of the data. All authors read and approved the final manuscript.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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References

1. Kos N, Gradisnik L, Velnar T. A brief review of the degenerative intervertebral disc disease. Med Arch. 2019;73:421–4. https://doi.org/10.5455/medarh.2019.73.421-424.
2. Lyu FJ, et al. Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions. Bone Res. 2021;9:7. https://doi.org/10.1038/s41413-020-00125-x.
3. Oichi T, Taniguchi Y, Oshima Y, Tanaka S, Saito T. Pathomechanism of intervertebral disc degeneration. JOR Spine. 2020;3: e1076. https://doi.org/10.1002/jsp2.1076.
4. Wang Y, et al. The role of IL-1beta and TNF-alpha in intervertebral disc degeneration. Biomed Pharmacother. 2020;131: 110660. https://doi.org/10.1016/j.biopha.2020.110660.
5. Kim H, Hong JY, Lee J, Jeon WJ, Ha IH. IL-1beta promotes disc degeneration and inflammation through direct injection of intervertebral disc in a rat lumbar disc herniation model. Spine J. 2021;21:1031–41. https://doi.org/10.1016/j.spinee.2021.01.014.
6. Xu H, et al. Reducing inflammation and vascular invasion in intervertebral disc degeneration via cystathionine-gamma-lyase inhibitory effect on e-selectin. Front Cell Dev Biol. 2021;9: 741046. https://doi.org/10.3389/fcell.2021.741046.
7. Effendi WI, Nagano T, Kobayashi K, Nishimura Y. Focusing on adenosine receptors as a potential targeted therapy in human diseases. Cells. 2020;9:785. https://doi.org/10.3390/cells9030785.
8. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. Nature. 2001;414:916–20.
9. Wu Q, et al. Electroacupuncture improves neuronal plasticity through the A2AR/cAMP/PKA signaling pathway in SNL rats. Neurochem Int. 2021;145: 104983. https://doi.org/10.1016/j.neuint.2021.104983.
10. Huang W, et al. An adenosine A1R–A2aR imbalance regulates low glucose/hypoxia-induced microglial activation, thereby contributing to oligodendrocyte damage through NF-κB and CREB phosphorylation. Int J Mol Med. 2018. https://doi.org/10.3892/ijmm.2018.3546.
11. Reis S, et al. Adenosine A1 and A2A receptors differently control synaptic plasticity in the mouse dorsal and ventral hippocampus. J Neurochem. 2019;151:227–37. https://doi.org/10.1111/jnc.14816.
12. Loram LC, et al. Intrathecal injection of adenosine 2A receptor agonists reversed neuropathic allodynia through protein kinase (PK)A/PKC signaling. Brain Behav Immun. 2013;33:112–22. https://doi.org/10.1016/j.bbi.2013.06.004.
13. Fozard JR, Ellis KM, Villela Dantas MF, Tigani B, Mazzoni L. Effects of CGS 21680, a selective adenosine A2A receptor agonist, on allergic airways inflammation in the rat. Eur J Pharmacol. 2002;438:183–8. https://doi.org/10.1016/s0014-2999(02)01305-5.
14. Rahimian R, et al. Adenosine A2A receptors and uric acid mediate protective effects of inosine against TNBS-induced colitis in rats. Eur J Pharmacol. 2010;649:376–81. https://doi.org/10.1016/j.ejphar.2010.09.044.
15. Basso DM, Beattie MS, Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp Neurol. 1996;139:244–56. https://doi.org/10.1006/exnr.1996.0098.
16. Yu L, et al. Genipin cross-linked decellularized nucleus pulposus hydrogel-like cell delivery system induces differentiation of ADSCs and retards intervertebral disc degeneration. Front Bioeng Biotechnol. 2021;9: 807883. https://doi.org/10.3389/fbioe.2021.807883.
17. Liu K, et al. Fexofenadine protects against intervertebral disc degeneration through TNF signaling. Front Cell Dev Biol. 2021;9: 687024. https://doi.org/10.3389/fcell.2021.687024.
18. Kamali A, et al. Small molecule-based treatment approaches for intervertebral disc degeneration: current options and future directions. Theranostics. 2021;11:27–47. https://doi.org/10.7150/thno.48987.
19. Zhang XB, et al. Targeted therapy for intervertebral disc degeneration: inhibiting apoptosis is a promising treatment strategy. Int J Med Sci. 2021;18:2799–813. https://doi.org/10.7150/ijms.59171.
20. Deng W, et al. Alcohol inhibits alveolar fluid clearance through the epithelial sodim channel via the A2 adenosine receptor in acute lung injury. Mol Med Rep. 2021. https://doi.org/10.3892/mmr.2021.12364.
21. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of adenosine receptors: the state of the art. Physiol Rev. 2018;98:1591–625. https://doi.org/10.1152/physrev.00049.2017.
22. Khoa ND, et al. Inflammatory cytokines regulate function and expression of adenosine A_{2A} receptors in human monocytic THP-1 cells. J Immunol. 2001;167:4026–32. https://doi.org/10.4049/jimmunol.167.7.4026.
23. Khoa ND, Postow M, Danielsson J, Cronstein BN. Tumor necrosis factor-alpha prevents desensitization of Galphas-coupled receptors by regulating GRK2 association with the plasma membrane. Mol Pharmacol. 2006;69:1311–9. https://doi.org/10.1124/mol.105.016857.
24. Castro CM, et al. Adenosine A2A receptor (A2AR) stimulation enhances mitochondrial metabolism and mitigates reactive oxygen species-mediated mitochondrial injury. Faseb j. 2020;34:5027–45. https://doi.org/10.1096/fj.201902459R.
25. Kwilasz AJ, et al. A single peri-sciatic nerve administration of the adenosine 2A receptor agonist ATL313 produces long-lasting anti-allodynia and

anti-inflammatory effects in male rats. Brain Behav Immun. 2019;76:116–25. https://doi.org/10.1016/j.bbi.2018.11.011.
26. Tsai CF, et al. The neuroprotective effects of an extract of *Gastrodia elata*. J Ethnopharmacol. 2011;138:119–25. https://doi.org/10.1016/j.jep.2011.08.064.
27. Dong W, et al. miR-640 aggravates intervertebral disc degeneration via NF-kappaB and WNT signalling pathway. Cell Prolif. 2019;52: e12664. https://doi.org/10.1111/cpr.12664.
28. Wu X, et al. Prolactin inhibits the progression of intervertebral disc degeneration through inactivation of the NF-kappaB pathway in rats. Cell Death Dis. 2018;9:98. https://doi.org/10.1038/s41419-017-0151-z.
29. Zhang GZ, et al. NF-kappaB signalling pathways in nucleus pulposus cell function and intervertebral disc degeneration. Cell Prolif. 2021;54: e13057. https://doi.org/10.1111/cpr.13057.
30. Sun K, et al. CGRP regulates nucleus pulposus cell apoptosis and inflammation via the MAPK/NF-kappaB signaling pathways during intervertebral disc degeneration. Oxid Med Cell Longev. 2021;2021:2958584. https://doi.org/10.1155/2021/2958584.
31. Li F, et al. Arginase II promotes intervertebral disc degeneration through exacerbating senescence and apoptosis caused by oxidative stress and inflammation via the NF-kappaB pathway. Front Cell Dev Biol. 2021;9: 737809. https://doi.org/10.3389/fcell.2021.737809.
32. Luo X, et al. Ulinastatin ameliorates IL-1beta-induced cell dysfunction in human nucleus pulposus cells via Nrf2/NF-kappaB pathway. Oxid Med Cell Longev. 2021;2021:5558687. https://doi.org/10.1155/2021/5558687.
33. Loram LC, et al. Enduring reversal of neuropathic pain by a single intrathecal injection of adenosine 2A receptor agonists: a novel therapy for neuropathic pain. J Neurosci. 2009;29:14015–25. https://doi.org/10.1523/jneurosci.3447-09.2009.
34. Yang Y, et al. Involvement of cAMP-PKA pathway in adenosine A1 and A2A receptor-mediated regulation of acetaldehyde-induced activation of HSCs. Biochimie. 2015;115:59–70. https://doi.org/10.1016/j.biochi.2015.04.019.
35. Masjedi A, et al. Downregulation of A2AR by siRNA loaded PEG-chitosan-lactate nanoparticles restores the T cell mediated anti-tumor responses through blockage of PKA/CREB signaling pathway. Int J Biol Macromol. 2019;133:436–45. https://doi.org/10.1016/j.ijbiomac.2019.03.223.
36. Harbrecht BG, et al. cAMP inhibits inducible nitric oxide synthase expression and NF-kappaB-binding activity in cultured rat hepatocytes. J Surg Res. 2001;99:258–64.
37. Zhao K, et al. Acid-sensing ion channels regulate nucleus pulposus cell inflammation and pyroptosis via the NLRP3 inflammasome in intervertebral disc degeneration. Cell Prolif. 2021;54: e12941. https://doi.org/10.1111/cpr.12941.
38. Zhao F, et al. Magnoflorine alleviates "M1" polarized macrophage-induced intervertebral disc degeneration through repressing the HMGB1/Myd88/NF-kappaB pathway and NLRP3 inflammasome. Front Pharmacol. 2021;12: 701087. https://doi.org/10.3389/fphar.2021.701087.
39. Meyers AK, Zhu X. The NLRP3 inflammasome: metabolic regulation and contribution to inflammaging. Cells. 2020. https://doi.org/10.3390/cells9081808.
40. Choi MC, Jo J, Park J, Kang HK, Park Y. NF-kappaB signaling pathways in osteoarthritic cartilage destruction. Cells. 2019. https://doi.org/10.3390/cells8070734.
41. Ishibashi K, et al. Adenosine A2A receptor occupancy by caffeine after coffee intake in Parkinson's disease. Mov Disord. 2022;37:853–7. https://doi.org/10.1002/mds.28897.
42. Citko A, Górski S, Marcinowicz L, Górska A. Sedentary lifestyle and nonspecific low back pain in medical personnel in North-East Poland. Biomed Res Int. 2018;2018:1965807. https://doi.org/10.1155/2018/1965807.
43. Huan H-C, et al. A closer examination of the interaction among risk factors for low back pain. Am J Health Promot. 2014;28:372–9. https://doi.org/10.4278/ajhp.120329-QUAN-171.
44. Ahn S, Song R. Bone mineral density and perceived menopausal symptoms: factors influencing low back pain in postmenopausal women. J Adv Nurs. 2009;65:1228–36. https://doi.org/10.1111/j.1365-2648.2009.04983.x.

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