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Attenuation of the progression of adjuvant-induced arthritis by 3-aminobenzamide treatment

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ABSTRACT

Rheumatoid arthritis (RA) is a disease that is still insufficiently controlled by current treatments. Poly(ADP-ribose) polymerase (PARP) inhibitors ameliorate immune-mediated diseases in several experimental models, including RA, colitis, experimental autoimmune encephalomyelitis and allergy. Together these findings showed that ADP-ribosylating enzymes, in particular PARP-1, play a pivotal role in the regulation of immune responses and may represent a noble target for new therapeutic approaches in immune-mediated diseases. The effect of 3-aminobenzamide (3-AB), an inhibitor of poly(ADP-ribose) synthetase activity, was evaluated in a mouse model of adjuvant-induced arthritis (AIA) on pro-inflammatory cytokines, adhesion molecules, inflammatory mediators and chemokine production/expression in serum and knee joint. Histopathological examination was also done on joint section. Our data demonstrates that 3-AB, 10 mg/kg, intraperitoneally (i.p.) significantly reduces pro-inflammatory cytokine (IL-17, TNF-α and IL-2) and chemokine (MCP-1 and MIP-2) production/expression, accompanied by amelioration of the disease as indicated by reduced paw swelling and arthritic scores and was associated with a significant reduction of VCAM-1 and ICAM-1 expression in the knee joint. Moreover, the expression of inflammatory mediators (iNOS, COX-2, MMP-2, MMP-9) and joint histological inflammatory damage was also markedly decreased. The results of this study suggest that PARP-1 inhibitor may play a role in the inflammatory arthritic process after administration of 3-AB may be a beneficial therapeutic approach.

1. Introduction

Poly(ADP-ribose) polymerase-1 (PARP-1) is a DNA binding protein that specifically detects DNA strand breaks or nicks and, using NAD^+ as a substrate, synthesizes and transfers ADP-ribose to several nuclear proteins. Different mechanisms have been proposed to explain the role of PARP-1 in the inflammatory response. The role of PARPs in inflammation has been investigated with pharmacological inhibitors and confirmed and/or better focused with gene specific deficient mice [1,2]. As shown in several experimental models, PARP inhibitors display protective effects in acute and chronic inflammatory diseases [3–7]. PARP-1 sustains the expression of cytokines, chemokines and other inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, interferon-γ (IFN-γ), CCL3 and inducible nitric-oxide synthase (iNOS). In mouse models, PARP inhibitors reduce gastric inflammation, prevent the T cell driven immunopathology and the formation of gastric precancerous lesions, and revert pre-existing lesions [8]. Preclinical studies also suggest a possible use of PARP-1 inhibitors in chronic inflammatory diseases, such as multiple sclerosis [9] and allergy/asthma [10].

Rheumatoid arthritis (RA) is a common, chronic inflammatory disease, characterized by intense, destructive infiltration of synovial tissue by a broad spectrum of inflammatory cells [11]. Multiple cytokines, derived from macrophages and fibroblasts, are responsible for induction and secretion of both cytokines and chemokines in RA [12]. More recently, IL-17 has been suggested to play an important additional key role in RA induction and maintenance [13,14]. Interleukin 17 is elevated in synovial fluids of RA patients and in inflamed joints of experimental arthritic mouse models [14,15]. IL-17 is a pro-inflammatory cytokine produced predominantly by T-helper-17 (Th17) cells [16,17]. IL-17 is an important regulator of immune and inflammatory responses, including the induction of other pro-inflammatory cytokines and osteoclastic bone resorption [13,18].

Various chemokines have been described in arthritic models. MIP-2, the murine equivalent of CXCL8/IL-8, has been detected in the joints of mice with collagen induced arthritis (CIA) in the early stage of disease, whereas the other chemokines like CCL5/RANTES and CCL3/MIP-1α were present in early as well as late disease stages [19]. In the rat AIA model it was shown that CCL3/MIP-1α and CXCL5/ENA-78-like proteins are involved in the onset of arthritis and joint erosion (JE); CCL2/MCP-1 is involved in late phases of the disease [20]. In arthritis, tissue

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destruction often correlates with the imbalance of matrix metalloproteinases (MMPs) over tissue inhibitors of metalloproteinase (TIMPs). In RA, synovial fluid contains higher levels of MMP-2 and MMP-9, both of which degrade the joint matrix because of their gelatinase activities [21].

Inducible nitric oxide synthase (iNOS), which catalyzes the oxidative deamination of L-arginine to produce NO, is responsible for prolonged and profound production of NO [22]. iNOS provoked deleterious consequences such as septic shock and inflammatory diseases [23]. Cyclooxygenase-2 (COX-2) is induced by several stimuli and is responsible for the production of large amounts of pro-inflammatory prostaglandins at the inflammatory site [24]. Vascular cell adhesion molecule-1 (VCAM-1) is strongly expressed on several cell types in RA synovium including fibroblast-like synovial lining and endothelial cells [25].

The aim of the present study was to investigate the effect of a PARP-1 inhibitor (3-AB) on murine AIA, which shares both immunological and pathological features with human RA. To illustrate the role of PARP-1 inhibitor in this model of RA, we determined the following endpoints of the RA response: paw edema, arthritic score and the levels of Th1 cytokines (IL-17A, TNF-α and IL-2) which were investigated using ELISA and RT-PCR in serum and knee joint. In addition we also investigated adhesion molecule (VCAM-1 and ICAM-1), inflammatory mediators (iNOS, COX-2, MMP-2 and MMP-9) and chemokines including macrophage inflammatory protein-1 (MIP-2) and monocyte chemotactic protein-1 (MCP-1) mRNA expressions in knee joint via RT-PCR. The effects of the treatment were confirmed by histological examination of knee joint section. The results of the current study supported the view that inhibition of 3-AB exerts significant anti-arthritic effects.

2. Materials and methods

2.1. Animals

Female adult Balb/c mice, 6–7 weeks old and weighing 20–25 g, were obtained from the animal house of the College of Pharmacy of King Saud University, Riyadh, KSA. The mice were maintained at 22 ± 2 °C with a 12 h light/dark cycle, housed in a specific pathogen-free environment and fed standard rodent chow and given water ad libitum. All procedures were performed with the approval of the Institutional Animal Care and Use Committee.

2.2. Chemicals

The compound 3-aminobenzamide (3-AB) was obtained from Santa Cruz Biotechnology, Inc. (USA). ELISA kits were obtained from Ray Biotech, Inc. (USA). Heparin was purchased from Sigma-Aldrich (USA). Complete fluid adjuvant (CFA) containing heat-killed *Mycobacterium tuberculosis* was obtained from Chondrex, Inc. (USA). The primers used in the current study for gene expression were purchased from Applied Bio Systems (UK) and Genscript (USA).

2.3. Induction of AIA and 3-AB treatment

Balb/c mice were induced with Complete Freund's Adjuvant (CFA) containing *M. tuberculosis*, a subplantar injection of 0.02 ml CFA containing 5 mg/ml of heat killed *M. tuberculosis* [26,30]. This arthritic model, called adjuvant-induced arthritis (AIA), has been widely used as a model for RA. 3-AB dissolved in DMSO was administered at a dose of (10 mg/kg, i.p.) once a day for 21 consecutive days after immunization. The dose of 3-AB (10 mg/kg, i.p.) was selected based on the results of the previous study [27,28]. The arthritic control (AC) and normal control (NC) groups were given saline only. Serum and knee joint samples were collected from all treated and control groups three weeks after the injection and knee joint samples were stored at − 70 °C until use. The measurement of the paw edema was performed using a volume displacement plethysmometer (Ugo Basile, Italy) which is the most commonly employed methods to measure the paw edema [29].

2.4. Measurement of the severity of arthritis

The mice were observed daily after the injection of CFA for the development of inflammatory arthritis until days 21. The severity of arthritis was blindly scored on a 0–4 scale/paw to avoid scoring biases: 0 = normal; 1 = mild erythema or swelling of the wrist or ankle or erythema and swelling of any severity for 1 digit; 2 = more than three inflamed digits or moderate erythema and swelling of the ankle or wrist; 3 = severe erythema and swelling inflammation of the wrist or ankle; and 4 = complete erythema and swelling of the wrist and ankle including all digits.

2.5. Estimation of IL-17, IL-2 and TNF-α cytokine levels on the serum

Serum samples from normal and arthritic controls as well as 3-AB treated groups were collected and immediately prepared for the analysis of cytokine levels. In protocols, commercially available kits were used with monoclonal specific antibodies for each cytokine. Cytokine levels were measured via ELISA using a kit from Ray Biotech, Inc. (USA) according to the manufacturer's instructions.

2.6. Reverse transcription-polymerase chain reaction (RT-PCR)

All extraction procedures were performed on ice using ice-cold reagents. Total RNA from mouse knee joint was isolated from homogenates using TRIzol reagent (Invitrogen), according to the manufacturer's instructions. The obtained mRNA was quantified by measuring the absorbance at 260 nm, and its quality was determined by measuring the 260/280 ratio. cDNA was synthesized using a high-capacity cDNA reverse transcription kit (Applied Biosystems) according to the manufacturer's instructions. Briefly, 1.5 μg of total RNA from each sample was added to a mixture of 2.0 μl of 10× reverse transcriptase buffer, 0.8 μl of 25× dNTP mix (100 mM), 2.0 μl of 10× reverse transcriptase random primers, 1.0 μl of multi-scribe reverse transcriptase and 3.2 μl of nuclease-free water. The final reaction mixture was held at 25 °C for 10 min, then heated to 37 °C for 120 min and 85 °C for 5 s and, finally, cooled to 4 °C.

2.7. Quantification of mRNA expression in knee joint via RT-PCR

Quantitative analysis of target gene mRNA expression was performed via RT-PCR by subjecting the cDNA obtained from the above preparation methods to PCR amplification in 96-well optical reaction plates using the ABI Prism 7500 System (Applied Bio systems). The 25 μl reaction mixture contained 0.1 μl of 10 μM forward primers and 0.1 μl of 10 μM reverse primers (40 μM final concentration of each primer), 12.5 μl of SYBR Green Universal Mastermix, 11.05 μl of nuclease-free water and 1.25 μl of the cDNA sample. The primers used in these assays were selected from PubMed and other databases, which are listed in Table 1. Assay controls were incorporated into the same plate, which consisted of no-template controls to test for contaminations of any of the assay reagents. The real-time PCR data were analyzed using the relative gene expression (i.e., ΔΔCT) method, as described in Applied Biosystems User Bulletin No. 2. Briefly, the data are presented as the fold change in gene expression normalized to an endogenous reference gene (GAPDH) and relative to a calibrator.

2.8. Histopathological examination

Mouse knee joints were removed 21 days after the injection of *M. tuberculosis*, then fixed for 4 days in 10% formalin, decalcified in decal solution (EDTA) in 5% formic acid, embedded in paraffin and sectioned (7 μm thickness) to determine the extent of joint