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Sulfasalazine Induces Apoptosis and Cell Cycle Arrest in RAW 264.7 Macrophages

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Sulfasalazine is a disease-modifying antirheumatic abiotic agent. It is a derivative of aminosalicylic acid and has been used for the treatment of various inflammatory diseases, such as rheumatoid arthritis, ulcerative colitis, and Crohn's disease, since it was first synthesized in 1941 and approved as a medicine in the United States in 1950. However, its mechanism of action has not yet been clearly identified. In this study, the effects of sulfasalazine on cell survival, apoptosis, and cell cycle progression in macrophages, which are major immune cells that regulate inflammatory responses, were investigated using mouse macrophage RAW 264.7 cells. Sulfasalazine inhibited the viability of RAW 264.7 cells in a dose-dependent manner, starting at a concentration of 0.25 mM. Annexin-V staining was used to confirm that the decrease in cell viability was due to apoptosis, and the number of Annexin-V-positive cells increased significantly at a concentration of 0.25 mM or higher. The effect of sulfasalazine on the expression of key proteins that regulate the G0/G1 phase of the cell cycle was also investigated. Sulfasalazine treatment significantly increased the expression of the cyclin-dependent kinase inhibitors p21 and p27 in RAW 264.7 cells. Although sulfasalazine is frequently used as a control drug in studies on inflammatory diseases, such as inflammatory colitis and rheumatoid arthritis, studies on its effect on macrophages are very limited. Therefore, the results of this study are expected to provide vital information on the use of sulfasalazine as a disease treatment.

Key words : Apoptosis, cell cycle arrest, macrophages, RAW264.7 cells, Sulfasalazine

Introduction

Inflammation is the body's defense mechanism against infection, injury and toxins. The inflammatory response is the process by which the immune system recognizes and eliminates harmful and external stimuli and initiates the healing process [2]. It is a complex process involving many different types of cells, including immune cells, and the substances that produce them [2, 13]. Chronic inflammation is slow, long-term inflammatory response that lasts for months or years. The degree and effects of chronic inflammation rely on the reason of the injury and the body's ability to fix and overcome the damage [2, 4, 9, 11]. Recently, studies showing that chronic inflammation mediates various diseases such as obesity, arteriosclerosis, type 2 diabetes, asthma, multiple sclerosis, rheumatoid arthritis, and cancer are gradually increasing [2, 4, 9, 11]. For this reason, the necessity of controlling the inflammatory response and the importance of anti-inflammatory agents for the treatment of various diseases are being emphasized.

Macrophages are representative immune cells that direct the overall inflammatory response and play an essential interface between innate and acquired immunity [27]. Stimulation of macrophages to internal and external stimuli leads to activation of various downstream signaling pathways. Among them, the transcription factor nuclear factor-κB (NF-κB) pathway is overwhelmingly important [2, 27]. Expression of key inflammatory mediators, including nitric oxide (NO) and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6, are driven by this pathway [2, 27]. Therefore, drugs that interfere with this signaling pathway are mainly used as anti-inflammatory agents.

Sulfasalazine was synthesized in 1942 by combining the antibiotic sulfapyridine with the anti-inflammatory drug 5-aminosalicylic acid (5-ASA) [25]. It is a safe drug that has been used as an antirheumatic agent for a long time and is used for the treatment of various inflammatory diseases such

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as rheumatoid arthritis and ulcerative colitis [18]. Sulfasalazine is decomposed into mesalazine and sulfapyridine by enteric bacteria, and it is known that these compounds have the main pharmacological action [19]. In addition, Smedegård et al., speculated that the effects of sulfasalazine in rheumatoid arthritis are probably mediated by various immunomodulatory and anti-inflammatory effects [23]. Sulfasalazine is still used centrally in the treatment of inflammatory bowel disease and rheumatoid arthritis, but how sulfasalazine achieves its therapeutic effects is not yet fully understood.

To elucidate the anti-inflammatory mechanism of sulfasalazine, experiments using human colorectal cancer cell lines and murine T cells revealed that sulfasalazine at millimolar concentration inhibited NF-κB activation [12, 26]. Other study demonstrated that sulfasalazine induced conversion of adenine nucleotides to adenosine by ecto-5'-nucleotidase in human microvascular endothelial cell line and its anti-inflammatory activities mediated by adenosine [14]. Compared to the existence of numerous experiments using sulfasalazine as a reference drug for inflammatory bowel disease or rheumatoid arthritis, studies observing various effects including anti-inflammatory action of sulfasalazine in macrophages are very limited. Therefore, in this study, the effects of sulfasalazine on cell viability, apoptosis and cell cycle progression in RAW264.7 cells, a murine macrophage cell line, were investigated.

Materials and Methods

Chemicals and Reagents

All reagents including lipopolysaccharide (LPS) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Sulfasalazine were obtained from Cayman Chemicals (Ann Arbor, MI). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin mixed solution were obtained from Hyclone (Logan, UT, USA). Z-VAD-FMK was purchased from Enzo Life Sciences (New York, NY). Celltiter-glo® luminescent substrate (G7570) were purchased from Promega Ltd (Madison, WI USA). Antibodies (Abs) used in this study included cyclin E (sc-481), cyclin dependent kinase 2 (CDK2, sc-748), CDK4 (sc-260), and p21 (sc- 397) obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA); cyclin D1 (#2978), p27 (#3688) and cleaved Poly (ADP-ribose) polymerase (PARP, #9541) were obtained from Cell Signaling Technology (Danvers, MA, USA); and GAPDH (MA5-15738) was obtained from Invitrogen (Waltham, MA, USA).

Cell culture and Sulfasalazine treatment

RAW264.7 cells were purchased from the Korea Cell Bank (Seoul, Korea) and maintained in DMEM supplemented with heat-inactivated FBS and penicillin-streptomycin mixed solution at 37℃ with 5% CO_2 in a humidified atmosphere. The cells attained 70-80% confluence with a normal morphology and multiplication pattern, and were used for experiments between passages 3-10. Sulfasalazine was dissolved in DMSO (250 mM) and filtered with 0.22 μm microfilter. Unless otherwise mentioned, control treatment refers to the cells treated with an equal amount of DMSO; the final concentration of DMSO in solution was less than 0.1%.

Measurement of nitrite and cytokines

RAW264.7 cells were plated at a density of 5×10^5 cells in a 24-well cell culture plate with 500 μl of culture medium and incubated for 12 hr. They were then treated with various concentrations of sulfasalazine in medium containing LPS (100 ng/ml) and incubated for an additional 24 hr. The amounts of nitrite and inflammatory cytokines were measured in cell culture supernatants using the Griess reagent system (Promega) and ELISA kits (eBioscience, San Diego, CA), respectively.

Cell viability assay

To evaluate the inhibitory effects of sulfasalazine on the cell viability of RAW264.7 cells, the CellTiter-Glo assay was performed according to the manufacturer's instructions. Briefly, RAW264.7 cells were plated and treated with sulfasalazine for 24 hr at concentrations of 0, 0.1, 0.25, 0.5, 1 and 2 mM in clear-bottomed, white 96-well plates. The CellTiter-Glo substrate (1:1) was added prior to the assay. The luminescence was measured using a GloMax 96 microplate luminometer (Promega).

Measurement of apoptotic cells

Apoptosis was assayed using a Muse™ Annexin V and Dead Cell Kit (Luminex, Austin, TX, USA) according to the manufacturer's user guide. RAW264.7 cells grown in 48-well plates (5×10^4 cells/well) were treated with sulfasalazine at different concentrations for 24 hr. Adherent cells were washed, collected, and incubated with Annexin V and 7-Aminoactinomycin D (7-AAD) for 20 min at room temperature in the dark. Percentages of live, dead, early, and late apoptotic cells were measured with a Muse® Cell Analyzer (Luminex).

Multi-Caspase Assay

To determine apoptosis by activation of multiple caspases

(caspase-1, -3, -4, -5, -6, -7, -8 and -9), the Muse™ Multi-caspase assay kit (Luminex) was used. RAW264.7 cells were treated with sulfasalazine at different concentrations for 24 hr. After harvesting the cells, cells were washed with caspase buffer. 5 μl Muse™ Multi-Caspase reagent working solution was added to the cells and incubated for 30 min at 37℃. 125 μl of Muse™ Caspase 7-AAD working solution was added in each tube and kept incubated for 5 min at room temperature. The data were detected using the Muse™ Cell analyzer (Luminex).

Cell cycle analysis

RAW264.7 cells were seeded in 24-well plate (1×10^5 cells/well) and incubated for 18 hr. Then, cells were treated with indicated concentrations of sulfasalazine for 24 hr. Cell cycle was analyzed by Muse cell analyzer using Muse Cell Cycle Kit (Luminex) according to manufacturer's instructions.

Western blot analysis

RAW264.7 cells (2×10^6 cells) grown in culture medium on 100 mm plates were treated with Sulfasalazine at indicated concentrations for 24 hr. Cells were washed with cold PBS and suspended with PRO-PREP™ protein extraction solution (Seongnam, Korea) containing a fresh mixture of complete protease and phosphatase inhibitors (Roche, Switzerland). The lysates were centrifuged (10,000 rpm/min, 10 min, 4℃). Then the supernatant was mixed with 4× sample buffer (Bio-Rad, Hercules, CA, USA) containing 10% β-mercaptoethanol and boiled for 5 min (100℃). The same amount of protein lysates was loaded and resolved in 8, 10, or 12% SDS-PAGE (100 V, 2 h). Separated proteins were transferred onto 45 μm PVDF Immobilon-P membrane (Merck Millipore, Burlington, MA, USA). The membrane was blocked with 3% bovine serum albumin (BSA, Sigma) for 1 h on the shaking incubator at room temperature. Target proteins were immunoblotted with indicated primary antibodies (1:1000 dilution) at 4℃ overnight and then incubated with an HRP-conjugated secondary antibody (1:5000 dilution) at 4℃ for 3 hr. Protein bands were then visualized using an enhanced chemiluminescent reagent (Thermo Fisher Scientific) and DAVINCH Chemi CAS-400SM (Davinch-k, Seoul, Korea). Protein levels were analyzed using a TotalLab analysis software ((Nonlinear Dynamics, Durham, NC, USA).

Statistical analysis

Values were expressed as the mean ± SEM of the results of at least three experiments. The values were then evaluated via one-way analysis of variance (ANOVA) with Bonferroni multiple comparison post-hoc tests using Prism 6.0 software (GraphPad Software, San Diego, CA, USA). Differences with p values < 0.05 were considered statistically significant.

Results

Effect of sulfasalazine on cell viability

To determine the effect of sulfasalazine on RAW264.7 cell viability, cells were treated with different concentrations of sulfasalazine for 24 hr. Sulfasalazine did not inhibit cell viability in RAW264.7 cells compared to untreated control cells up to concentrations of 0.1 mM. However, at subsequent concentrations, it significantly inhibited cell viability in a dose-dependent manner.

Effect of sulfasalazine on production of inflammatory mediators in LPS-stimulated RAW264.7 cells

We first investigated the inhibitory effect of sulfasalazine on the production of inflammatory mediators, NO and proinflammatory cytokines. At concentrations above 0.1 mM, sulfasalazine significantly inhibited LPS-induced NO production as shown in Fig. 2A. In cells exposed to LPS, the inhibitory effect of sulfasalazine on IL-6 production was similar to that on NO production (Fig. 2B). In contrast, sulfasalazine did not inhibit the production of TNF-α induced by LPS (Fig. 2C). These results confirmed that sulfasalazine exhibited anti-inflammatory activity by inhibiting NO and IL-6 production

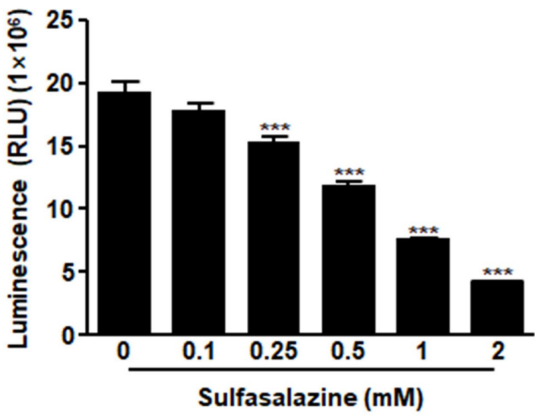

Fig. 1. Effect of sulfasalazine on cell viability in RAW264.7 cells. Cell viability of RAW264.7 cells treated with the indicated concentrations of sulfasalazine was assessed using CellTiter-Glo assay at 24 hr. The results are expressed as mean ± SEM of three independent experiments. Statistical significance is based on the difference when compared with control cells (0 mM) ($^{***}p<0.001$).

in inflammatory conditions.

Effect of sulfasalazine on induction of apoptosis in RAW264.7 cells

Based on the result that sulfasalazine treatment reduced the viability of RAW264.7 cells, the possibility that sulfasalazine induces apoptosis was investigated. At the lowest concentration of 0.25 mM, which showed a cell survival in-

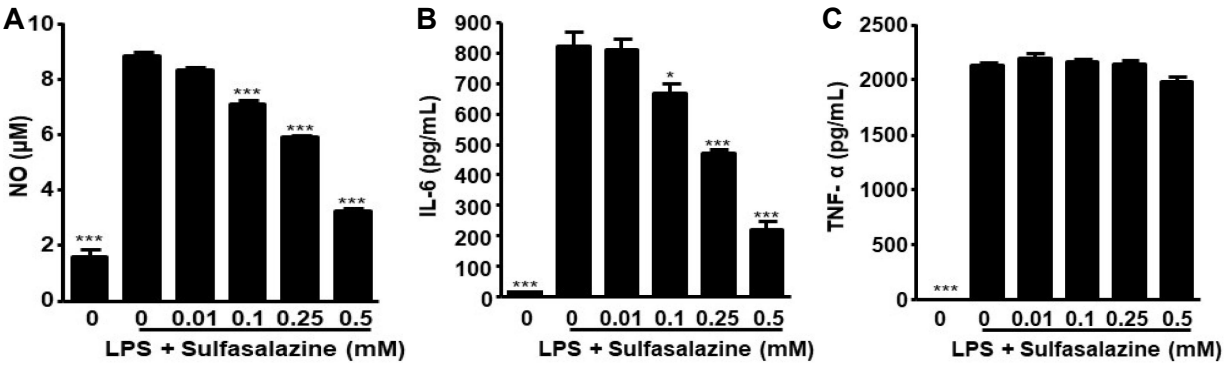

Fig. 2. Effects of sulfasalazine on LPS-induced NO and inflammatory cytokines releases. RAW264.7 cells were treated with indicated concentrations of sulfasalazine in the presence of 100 ng/ml of LPS or with LPS alone for 24 hr, and (A) NO, (B) IL-6 and (C) TNF-α release was determined. The results are reported as mean ± SEM of three independent experiments in triplicate. Statistical significance is based on the difference when compared with LPS-stimulated cells ($^{*}p<0.05$, $^{***}p<0.001$).

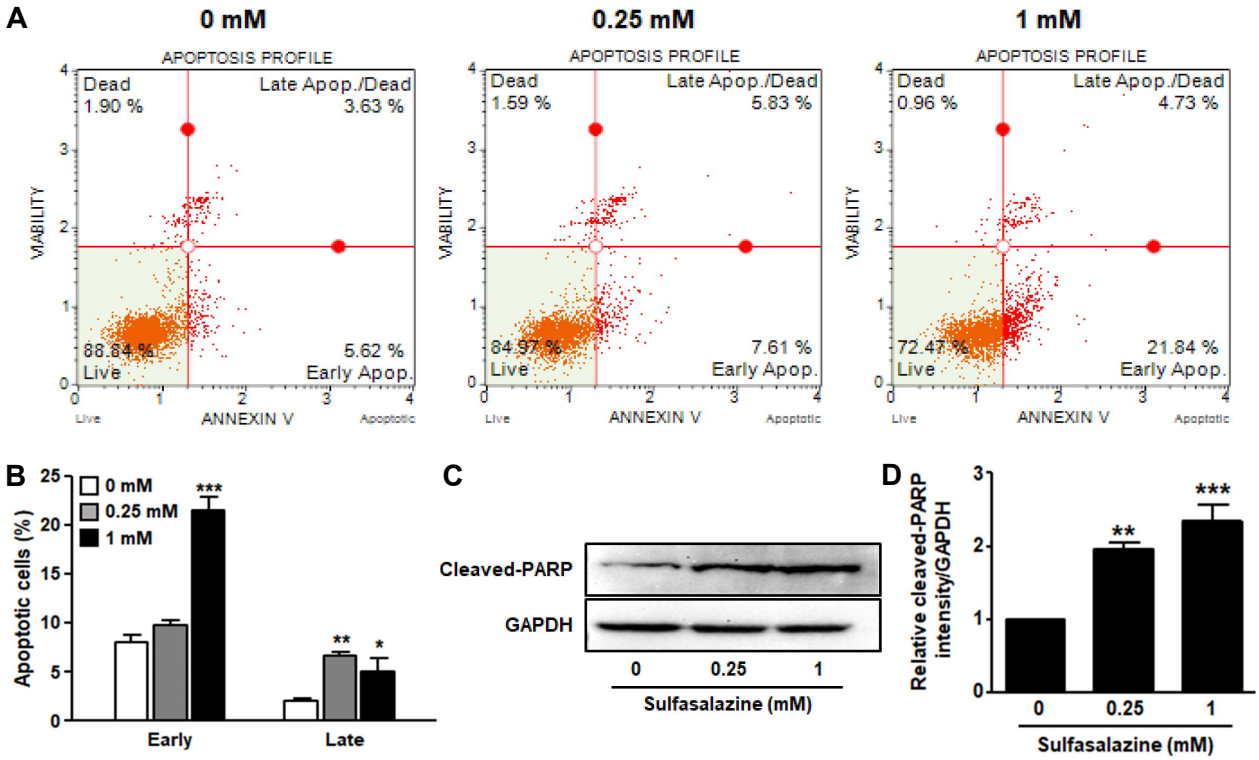

Fig. 3. Apoptotic effects of sulfasalazine on RAW264.7 cells. (A) RAW264.7 cells were treated with the indicated concentrations of sulfasalazine for 24 hr, stained with Muse Annexin V & Dead Cell reagent and then analyzed for apoptosis by Muse Cell Analyzer. (B) The percentage of cells undergoing apoptotic cell death is presented as the mean ± SEM from three separate experiments. (C) Representative bands of cleaved PARP from the western blot analysis. GAPDH was used as a loading control. (D) Densitometry analysis of cleaved PARP in sulfasalazine-treated RAW264.7 cells detected with Western blot. Statistical significance is based on the difference when compared with control cells (0 mM) ($^{*}p<0.05$, $^{***}p<0.001$).

hibitory effect in Fig. 1B, the percentage of late apoptotic cells was significantly increased. Also, there was a significant increase in early and late apoptotic cells at a concentration of 1 mM (Fig. 3A, Fig. 3B). In addition, the level of cleaved PARP was increased by sulfasalazine treatment for 24 hr (Fig. 3C, Fig. 3D).

It is known that caspase activation is correlated with the onset of apoptosis and that caspase inhibition can attenuate apoptosis. Therefore, the involvement of caspases in sulfasalazine-induced apoptosis in RAW264.7 cells was investigated using multiple caspases analysis. Activities of multiple caspases (caspase-1, -3, -4, -5, -6, -7, -8, -9) were measured using a Muse™ Cell Analyzer (Fig. 4A, Fig. 4B). Total multi-caspase activities in RAW264.7 cells treated with 0, 0.25, and 1 mM sulfasalazine were 22.92±0.95%, 26.6±2.53%, and 35.6±2.73%, respectively. In contrast, live cells in RAW 264.7 cells treated with 0, 0.25, and 1 mM sulfasalazine were 75.32±0.12%, 72.7±2.64%, and 63.3±2.46%, respectively.

Next, to investigate the contribution of caspases to sulfasalazine-mediated cell death, RAW264.7 cells were treated with the pan-caspase inhibitor Z-VAD-FMK together with sulfasalazine. The percentage of viable cells was significantly reduced to 60% at 1 mM sulfasalazine concentration. However, as shown in Fig. 4C, inhibiting caspases significantly improved cell survival in sulfasalazine-treated RAW264.7 cells. These results suggest that caspases contribute to sulfasalazine-mediated cell death.

Effect of sulfasalazine on cell cycle progression in RAW264.7 cells

Finally, cell cycle progression was investigated to determine whether the decrease in cell viability induced by sulfasalazine was due to impairment of cell cycle progression. Results are demonstrated in Fig. 5. Compared with the control (0 mM treated cells), sulfasalazine significantly increased G0/G1 cells and decreased the proportion of S- and G2/M-phase cells as shown in Fig. 5A and Fig. 5B. We found that the G0/G1 phase increased from 55.6 ± 1.80% in control to

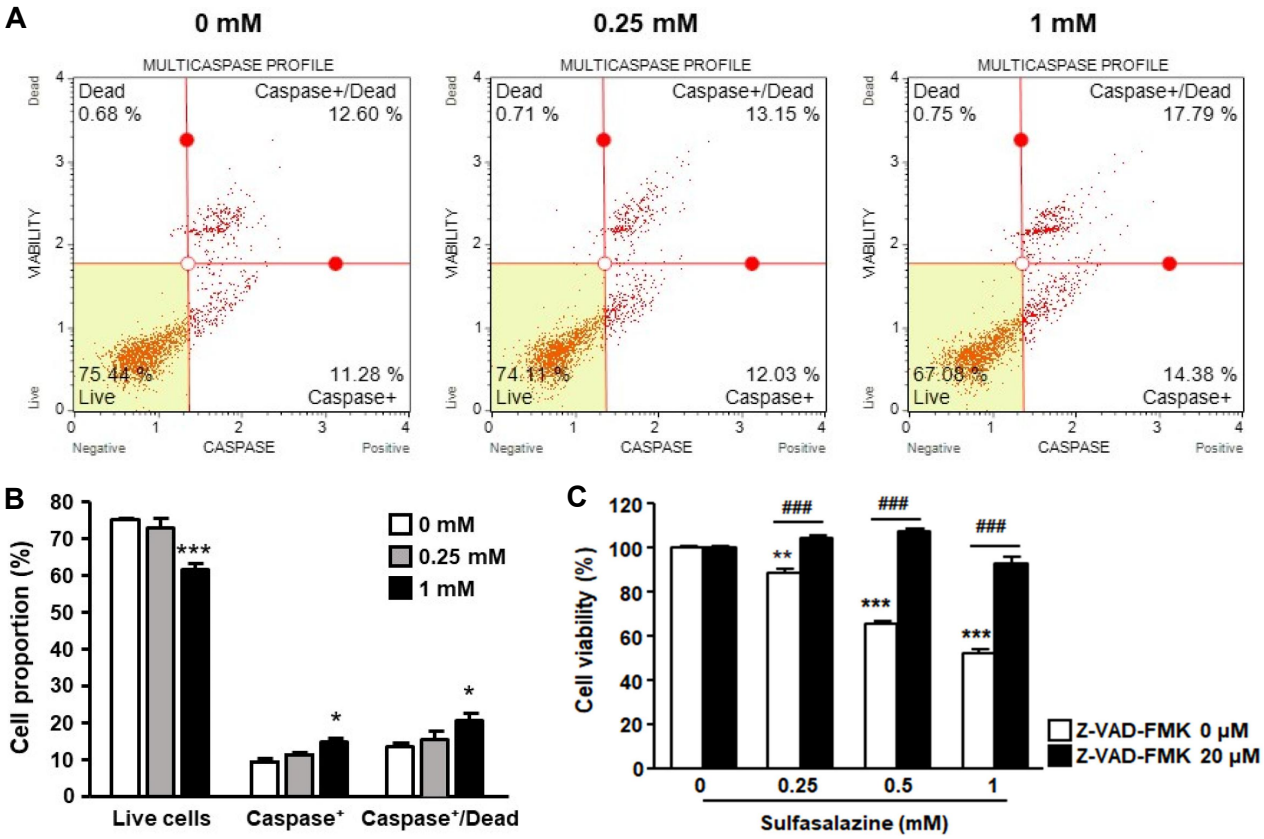

Fig. 4. Effect of sulfasalazine on the expression of caspases activity. Cells were treated with sulfasalazine for 24 hr. (A) Multi-caspase activity was measured with the Muse™ Multi-caspase Kit. (B) Quantification of total caspase activation. (C) RAW264.7 cells were treated with different concentrations of sulfasalazine and Z-VAD-FMK (20 μM) for 24 hr. The results are expressed as mean ± SEM of three independent experiments. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs. control cells; $^{\#\#\#}p<0.001$ vs. sulfasalazine treatment in the absence of Z-VAD-FMK cells.

64.53 ± 2.22% and 71.01 ± 1.45% in RAW264.7 cells treated with 0.25 and 1 mM sulfasalazine, respectively (Fig. 5B). Additionally, similar to the Annexin-V staining results, a significant increase in Sub-G1 was observed in the 1mM sulfasalazine treated group.

Since sulfasalazine significantly increased the proportion of cells in the G0/G1 phase in RAW264.7 cells, we next examined the effects of sulfasalazine on the expression of G0/G1 phase-associated proteins, including cyclin D1, cyclin E, CDK4, CDK2, p21 and p27 using western blot analysis. The expression of CDK4, which is responsible for the early stage of the G0/G1 phase, was significantly decreased at 1 mM of sulfasalazine, but the expression of Cyclin D1 was increased as shown in Fig. 6A and Fig. 6B. On the other hand, sulfasalazine treatment did not affect the expression of CDK2 and cyclin E, which regulate the progression from

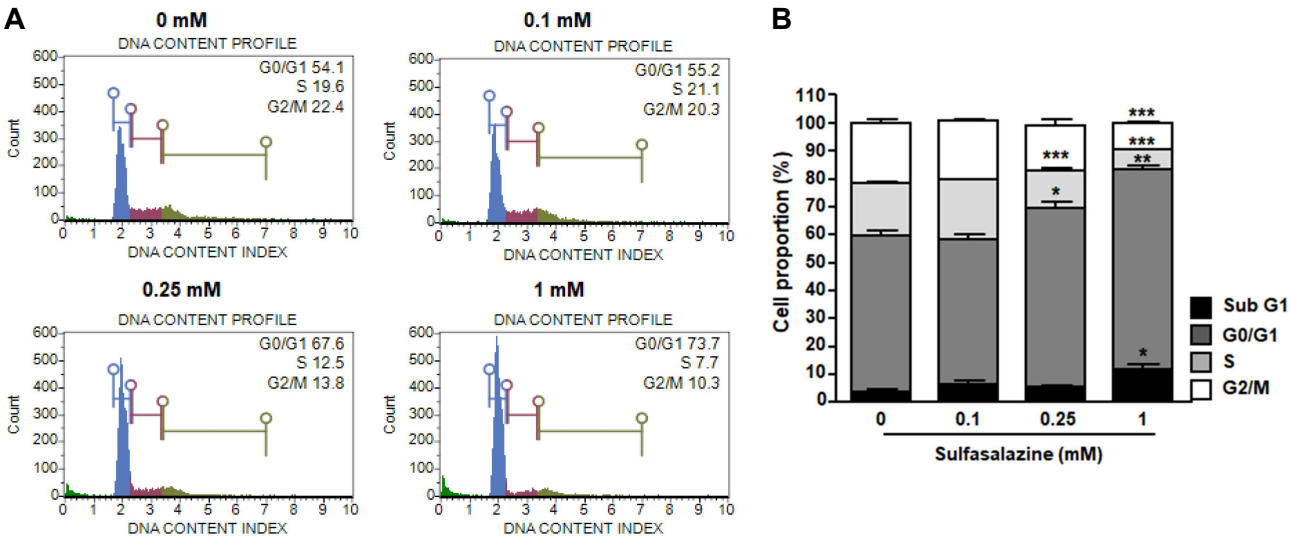

Fig. 5. Effect of sulfasalazine on cell cycle progression in RAW264.7 cells. (A) RAW264.7 cells were treated with the indicated concentration of sulfasalazine for 24 hr and subjected to a cell cycle assay using the Muse Cell Analyzer. (B) The statistical analysis of cell cycle distribution. The results are reported as mean ± SEM of three independent experiments. Statistical significance is based on the difference when compared with control cells (0 mM) ($^{*}p<0.05$, $^{***}p<0.001$).

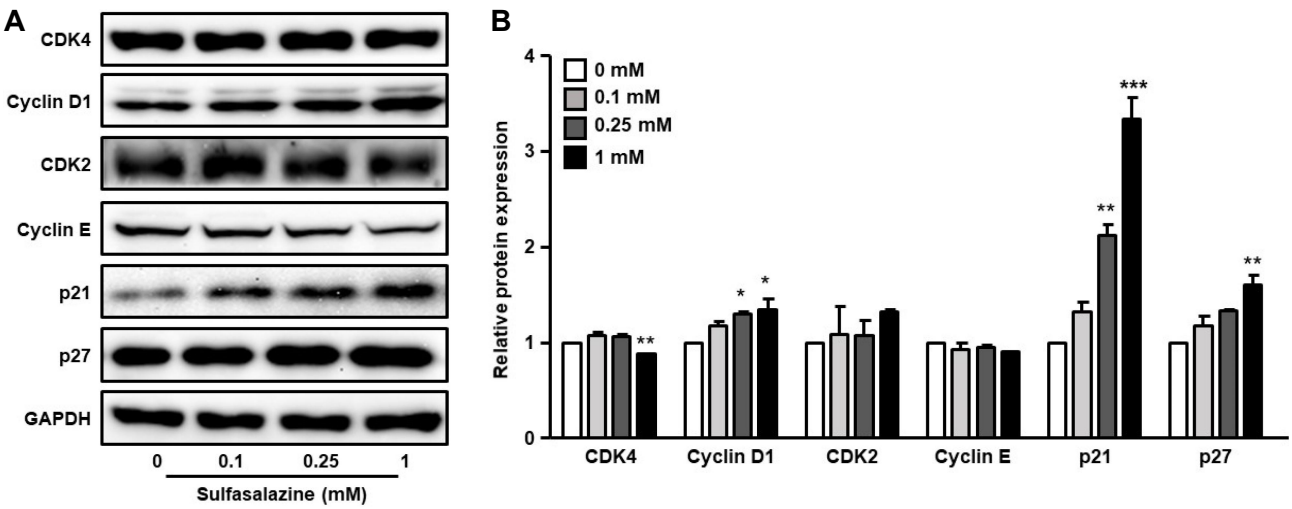

Fig. 6. Effect of sulfasalazine on the expression of cell cycle regulatory proteins in RAW264.7 cells. Cells were cultured with various doses of sulfasalazine for 24 hr. (A) Representative bands of CDK4, Cyclin D1, CDK2, Cyclin E, p21 and p27 from the western blot analysis. GAPDH was used as a loading control. (B) Densitometry analysis of CDK4, Cyclin D1, CDK2, Cyclin E, p21 and p27. Statistical significance is based on the difference when compared with control cells (0 mM) ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

G1 to S phase. The most notable changes by sulfasalazine treatment were observed in the CDK inhibitors p21 and p27. Significant increases in p21 were observed in cells treated with 0.25 mM and 1 mM sulfasalazine, and p27 in cells treated with 1 mM sulfasalazine. These results indicated that the inhibition of cell proliferation by sulfasalazine may be associated with the induction of G0/G1 phase arrest.

Discussion

Sulfasalazine is a US Food and Drug Administration-approved disease-relieving antirheumatic drug commonly used in the treatment of rheumatoid arthritis and inflammatory bowel disease, first used in 1940 for rheumatoid polyarthritis [16]. Recently, many studies suggest that sulfasalazine can be a modulator of various diseases, including cancer, due to its immunomodulatory and antiproliferative properties in addition to its anti-inflammatory effect [8, 16, 28, 32]. Because of its broad range of effects, it offers promise as a modulator of various diseases, but the level of evidence supporting its efficacy and safety is limited. More research is needed to fully elucidate the pharmacological potential of sulfasalazine. Therefore, in this study, the effects of sulfasalazine on cell proliferation, apoptosis, and cell cycle progression were investigated using RAW 264.7 cells to provide basic data for drug application of sulfasalazine.

Studies on the effect of sulfasalazine on apoptosis of immune cells, especially macrophages, are very rare. In human neutrophils, sulfasalazine significantly accelerated insoluble immune complex-induced apoptosis mediated by caspase 8 and caspase 3 activation, but not a caspase 9-dependent pathway [1]. Sulfasalazine also induced apoptosis in the mouse T cell line, RBL5 T lymphocytes. As a result of treating RBL5 cells with 2.5 mM sulfasalazine, more than 90% of the cells were observed as annexin V-positive cells after 8 hours, suggesting that sulfasalazine induces apoptosis in T lymphocytes as well [12]. In addition, Salh et al., showed that treatment of RAW 264.7 cells with 10 mM sulfasalazine for 6 hours induced apoptosis with caspase 3 activation and PARP cleavage [21]. They also demonstrated that treatment with 1 mM sulfasalazine for 24 hr or treatment with 0.5 mM sulfasalazine for 48 hours could induce apoptosis while reducing procaspase-3. Similar to these reports, our results also showed that treatment with 1 mM sulfasalazine for 24 hr significantly increased the activity of various caspases, early and late apoptotic cells. Combining the results of this study with those of previous studies, sulfasalazine is estimated to induce apoptosis of various cells such as macrophages, immune cells, and various cancer cells, although the concentration and incubation times to induce apoptosis varies depending on the type of cell.

Several studies have reported that sulfasalazine induced apoptosis in various cancer cells and shows potential as an anti-tumor agent using these properties [6, 15, 20]. Robe et al showed that apoptosis was induced in human glioblastoma cells, LN18 and U87 cells, after treatment with 1 mM sulfasalazine [20]. In addition to inducing apoptosis, sulfasalazine was found to induce ferroptosis by inhibiting xCT [6, 29, 30]. Ferroptosis is a recently discovered form of regulated cell death that is morphologically, genetically, and biochemically distinct from apoptosis and necroptosis, and its potential use in anticancer therapy is emerging [3, 31]. Ferroptosis is characterized by the accumulation of toxic reactive oxygen species and lipid peroxidation products derived from iron metabolism [3, 31]. xCT is a functional subunit of cystine/glutamate antiporters (system xc^-) that regulates the exchange of glutamate and cystine in cells [7]. Sulfasalazine has been shown to be an inhibitor of the system xc^- transporter, and disruption of system xc^--mediated cystine uptake by sulfasalazine naturally leads to induction of ferroptosis [5, 22]. Indeed, many subsequent studies demonstrated that sulfasalazine exhibited antitumor activity by inducing ferroptosis in esophageal cancer cells, breast cancer cells, and uterine serous carcinoma [24, 29, 30]. As we did not investigate whether sulfasalazine induces ferroptosis in RAW264.7 cells in this study, we plan to investigate this in a future study.

Contrary to many findings that sulfasalazine induces apoptosis or ferroptosis, there are few reports on the effect of sulfasalazine on cell cycle progression. Nagane et al., showed that sulfasalazine inhibited cellular DNA damage repair and induced cell cycle arrest at the G0/G1 phase after X-irradiation in murine B16F10 melanoma cells [17]. Cell cycle arrest was also observed in the G0/G1 phase of vascular smooth muscle cells, which was mediated by induction of haem oxygenase-1 followed by an increased expression of p21 [10]. Similar to these previous findings, we showed when RAW 264.7 cells were treated with sulfasalazine in the range of 0.25 mM to 1 mM, the expression of p21 and p27 was significantly increased, resulting in cell cycle arrest in the G0/G1 phase. Since treatment with sulfasalazine had no effect on the expression of cyclin E and CDK2, it seems that sulfasalazine inhibits cell cycle progression in the early G1 phase rather than affecting the late G1 phase. Furthermore, sulfasalazine-induced cell cycle arrest in RAW264.7 cells is probably

driven by p21 and p27. This study is, to the best of our knowledge, the first report that sulfasalazine caused cell cycle arrest in RAW264.7 cells.

Chronic inflammation has been found to be a major culprit in a variety of health conditions, including cardiovascular disease, diabetes mellitus, dementia, asthma, obesity and age-related macular degeneration. Therefore, the use of anti-inflammatory agents that control inflammation is important for the regulate of various diseases. In this study, it was revealed that sulfasalazine, a representative anti-inflammatory drug, can induce apoptosis and cell cycle arrest in macrophages, a representative immune cell that regulates the inflammatory response, and these results are expected to provide basic information for the use of sulfasalazine.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 마우스 대식세포에서 설파살라진의 세포사멸 및 세포주기 정체에 미치는 영향 연구

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설파살라진은 1941년 최초로 합성된 이후, 류마티스 관절염, 궤양성 대장염 및 크론병을 치료하는 데 사용되는 질병 변형 항 류마티스 약물-비 생물제제 (아미노살리실산 유도체)이다. 1950년 미국에서 의약품으로 승인된 이후 다양한 염증성 질환의 치료제로 사용되고 있으나 이 약물의 작용기전은 아직 명확하게 밝혀지지 않고 있다. 본 연구에서는 설파살라진이 염증반응을 조절하는 주요 면역세포인 대식세포의 세포생존, 세포사멸 및 세포주기 진행에 어떠한 영향을 미치는지를 마우스 대식세포인 RAW264.7 세포를 이용하여 조사하였다. 세포생존에 미치는 설파살라진의 영향을 측정한 결과 농도의존적으로 RAW264.7 세포의 생존을 억제하였다. 이러한 세포생존의 억제가 세포사멸에 기인한 것인지를 확인하기 위해 Annexin-V로 염색한 결과 0.25 mM 이상의 농도에서 Annexin-V 양성세포가 유의적으로 증가하였다. 또한 0.25 mM 이상의 농도에서 G0/G1기에서 유의적으로 세포주기 정체를 유도하는 것을 확인할 수 있었다. G0/G1기를 조절하는 주요 단백질의 발현을 확인한 결과 설라살라진의 처리는 RAW264.7 세포에서 CDK의 억제제인 p21과 p27의 단백질 발현을 유의적으로 증가시켜 설라살라진에 의한 G0/G1기의 정체는 p21과 p27에 기인하는 것으로 사료된다. 염증성 궤장염이나 류마티스 관절염과 같은 염증성 질환에서 설라살라진이 대조약으로 빈번하게 사용되어지고 있음에도 불구하고 대식세포에 미치는 영향에 대한 연구가 매우 제한적이어서 본 연구의 결과가 질병치료제로서의 설파살라진의 이용에 기초적인 정보를 제공할 수 있을 것이라 판단된다.