The Endocannabinoid, Anandamide, Acts as a Novel Inhibitor of LPS-Induced Inflammasome Activation in Human Gastric Cancer AGS Cell Line: Involvement of CB1 and TRPV1 Receptors

Sahar Sadat Sedeighzadeh,1 Hamid Galehdari,1,2 Mohammad Reza Tabandeh1,2,3 Mehdi Shamsara4, and Ali Roohbakhsh5

1Department of Biological Sciences, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz 61357-831351, Iran
2Stem Cells and Transgenic Technology Research Center, Shahid Chamran University of Ahvaz, Ahvaz 61357-831351, Iran
3Department of Basic Sciences, Division of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz 61357-831351, Iran
4Department of Animal Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran 14155-6343, Iran
5Department of Pharmacodynamics and Toxicology, Mashhad University of Medical Sciences, Mashhad 91388-13944, Iran

Correspondence should be addressed to Mohammad Reza Tabandeh; m.tabandeh@scu.ac.ir

Received 22 October 2020; Revised 15 February 2021; Accepted 25 February 2021; Published 13 March 2021

Copyright © 2021 Sahar Sadat Sedeighzadeh et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Inflammasome activation is a pivotal step for the maturation of IL-1β, which is involved in the development and progression of gastric cancer (GC). Endocannabinoids, such as anandamide (AEA), are emerging as new anticancer therapeutic agents; however, their effects on inflammasome components and underlying mechanisms have not been well elucidated. This study was designed to investigate the effects of AEA on the expression of inflammasome components in lipopolysaccharide- (LPS-) stimulated AGS cells. Moreover, we explored the involvement of cannabinoid receptors (CRs), including CB1R and TRPV1R, in the observed effects of AEA. Our results showed that inflammation was induced by LPS (10 μg/ml) in AGS cells, and inflammasome components (NLRP3, NLRC4, ASC, IL-18, and IL-1β) were overexpressed. Exposure to AEA (10 μM, 24 h) before or after inflammation induction downregulated the expression of inflammasome components and attenuated inflammasome activation as demonstrated by cleavage of caspase 1 and matured IL-1β secretion, although AEA pretreatment showed more reducing effects on the inflammasome activation. In addition, blocking of CB1R and TRPV1R by application of AM-251 and AMG-9810 antagonists remarkably reversed the observed effects of AEA and revealed that NLRP3, NLRC4, and IL-1β genes were mainly regulated via CB1R, while TRPV1R could only regulate the expression of IL-1β and IL-18 genes. In conclusion, our results would indicate a novel anticancer effect of anandamide by attenuation of inflammasome activation and consequently reducing IL-1β production in human AGS cancer cell line via CB1R and TRPV1R.

1. Introduction

Gastric cancer (GC) is the fourth frequent malignancy and the second cause of cancer-related death worldwide [1, 2]. Chronic inflammation has been accepted as one of the main causes for development and progression of GC [3]. Stomach infections, such as Helicobacter pylori, and chronic gastritis present strong etiological links between inflammation and gastric tumorigenesis [3]. Inflammasomes are intracellular protein complexes that drive inflammatory reactions in response to harmful stimuli such as pathogens. Inflammasomes are made up of NLR family pyrin domain-containing 1 (NLRP1), NLRP3, NLR family CARD domain-containing 4 (NLRC4), and absent in melanoma 2 (AIM 2) which consider as inflammasome sensor molecules, apoptosis-associated speck-like protein (ASC) in the role of an adaptor protein, and caspase 1 as an end activator of inflammasome pathway [4]. Upon these protein complexes formed,
inflamasome switches caspase 1 to its active form and cleaves proinflammatory cytokines, including interleukin-1β (IL-1β) and IL-18, to their mature forms [4].

IL-1β not only is expressed highly in GC tissues but also correlates with clinical and pathological features of GC [5]. Stomach-specific overexpression of human IL-1β in transgenic mice leads to spontaneous gastric inflammation and cancer [5]. Increased levels of IL-1β are believed to contribute to the signs of inflammation such as the development of severe gastritis [6]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are mostly used to alleviate inflammation [7]. Despite their effectiveness, NSAID treatment may induce severe side effects such as gastrointestinal damage and respiratory abnormalities [8]. More recently, there has been increasing interest in finding more compatible compounds to alleviate gastric inflammation [9, 10].

Endocannabinoids are a group of lipid neurotransmitters found in several tissues [11]. They are a subclass of cannabinoids, the active compounds of *Cannabis sativa* [11]. N-Arachidonoylthanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) are two main endocannabinoids described to now. Since the 1970s, when it was found that cannabinoids inhibited Lewis lung adenocarcinoma growth, the anticancer effects of these compounds have received increasing attention [12, 13]. Cannabinoids usually act via two distinct receptors including CB1 and CB2 receptors (CB1R and CB2R). CB1Rs are predominantly found in the peripheral and central nervous system, and CB2Rs are mostly expressed in the immune cells [14]. AEA can bind and activate both CB1 and CB2 receptors, but shows less intrinsic activity when binding CB2 receptor [15, 16]. In recent years, a great deal of evidence has been shown that anandamide also activates the transient receptor potential type 1 receptor (TRPV1R) [17]. Recent investigations have shown that cannabinoid agonists induced cancer cell apoptosis [18] and enhanced anti-inflammatory cytokine production [19]. Nevertheless, the underlying molecular mechanisms in GC are still not fully understood.

Up to now, there is no data available about the involvement of endocannabinoids and inflamasome in GC. In light of these data, to identify the relationship among endocannabinoid system, inflamasome, and IL-1β secretion, this study was designed to characterize whether and how anandamide can influence inflamasome component transcription and IL-1β secretion in AGS cell line. Our results might provide more evidence for the therapeutic value of endocannabinoid-based therapies for the treatment of GC in the future.

2. Materials and Methods

2.1. Cell Line, Culture Conditions, and Preparation of Chemical Regents. The AGS human GC cell line was obtained from Pasteur Institute Cell Bank (Tehran, Iran) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin (Gibco, Carlsbad, CA, USA) in a CO2 incubator at 37°C with 99% humidity and 5% CO2. The cells were starved 6 h with serum-free RPMI-1640 before treatment, and all treatments except LPS and ATP induction were performed in RPMI-1640 medium supplemented with 2% FBS. AEA (Sigma-Aldrich, MO, USA) was dissolved in absolute ethanol and made aliquots in dark microtubes and stored at -20°C. AM-251 and AMG-9810 (Tocris Bioscience, Bristol, UK) were dissolved in an absolute ethanol and stored in a refrigerator (2-8°C).

2.2. LPS-Induced Inflammation in AGS Cells. Inflammation was induced using LPS (*Escherichia coli* O111:B4; Sigma-Aldrich, MO, USA) as described previously [20]. Briefly, AGS cells were seeded in a 6-well plate at a density of 7 x 10^5 cells/well and cultured overnight; then, they were induced with LPS at the dose of 10 μg/ml for 6 h and treated with ATP (Honeywell Fluka™, Shanghai, China) at the final concentration of 5 mM for 30 min.

2.3. Cell Viability Assays. The viability of AGS cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. AGS cells were cultured in 96-well culture plates (8 x 10^3 cells/well) in RPMI-1640 medium. The cells from the overnight culture were treated with LPS (10 μg/ml) for 6 h and various concentrations of AEA (2.5-40 μM) (Sigma-Aldrich, MO, USA) for 12 and 24 h. Ten microliters of MTT solution (5 mg/ml) (Sigma-Aldrich, MO, USA) was added to each well; after incubation at 37°C for 3 h, the medium was replaced with 100 μl of dimethyl sulfoxide (DMSO, Wako Pure Chemical Industries, Ltd.). The absorbance of all wells was measured at 570 nm using a microplate reader (BioTek, USA). Data were displayed as the percentage of viable cells in treated groups compared to vehicle-treated cells and resulted from three independent experiments with triplicated wells.

2.4. Experimental Design. AGS cells were seeded at a density of 7 x 10^5 cells/well in 6-well plates and divided into four groups including control, LPS, pretreatment (RT), and post-treatment (OT) groups. The blank control cells were cultured in RPMI-1640 medium and treated only with ATP (5 mM, 30 min). Cells in LPS control group were treated with LPS (10 μg/ml, 6 h) and ATP (5 mM, 30 min). In RT group, the cells that had been treated with 10 μM AEA for 24 h induced with LPS (10 μg/ml, 6 h) and ATP (5 mM, 30 min), and the cells in OT group were first treated with LPS (10 μg/ml, 6 h) and ATP (5 mM, 30 min) and then treated with AEA (10 μM, 24 h). At the end of experiments, cell-free supernatants and cell pellets were collected and stored at -70°C for subsequent analyses. Schematic diagram of our experimental protocol is shown in Figure 1.

2.5. Blocking of CB1 and TRPV1 Receptors in AEA Treated AGS Cells. To blockade the cannabinoid receptors, AGS cells were pretreated with CB1R (AM-251) or TRPV1 (AMG-9810) antagonists. The efficient concentrations of these antagonists were selected based on the amount of IL-1β secretion in the culture media after pretreatment with various concentrations of AM-251 and AMG-9810 in AEA-stimulated cells using ELISA method. Accordingly, a concentration of 1 μM of AMG-9810 and AM-251 was selected for subsequent experiments. Because of the greater effect of
AEA on inflammasome components in RT group, inhibition of cannabinoid receptors was conducted only in this experimental group. Briefly, the cells were seeded in 6-well plates and pretreated with AM-251 (1 μM, 1 h) and AMG-9810 (1 μM, 30 min). Following AEA treatment (10 μM, 24 h) and LPS induction, cell-free supernatants and cell pellets were collected and stored at -70°C for subsequent analysis.

2.6. RNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR (qRT-PCR). Total cellular RNA was extracted using TriPure Isolation Reagent (Roche, Basel, Switzerland) according to the manufacturer’s procedure. All extractions were followed by DNase I (Thermo Fisher Scientific, Massachusetts, USA) treatment to remove contaminating genomic DNA. First-strand cDNA was synthesized from 2 μg RNA using Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc, Massachusetts, USA) with oligo (dT) and random hexamer primers according to manufacturer’s protocol. Afterward, cDNA was amplified for qRT-PCR by the qPCR™ Green Master Kit for SYBR Green I® (Yektatajhiz, Tehran, Iran) using specific primers (Table 1). The relative expression level of the target genes was compared to beta2-microglobulin (B2M) as a housekeeping gene. All reactions were performed in triplicate. Relative quantification was performed according to the comparative \(2^{-\Delta\Delta C_t}\) method. Validation of assay to check that the primer for target genes and housekeeping gene had similar amplification efficiencies was performed as described previously [21].

2.7. Western Blot Analysis. Total proteins were extracted from cells using RIPA lysis buffer (Tris-HCl 50 mM, NaCl 150 mM, Triton X-100 0.1%, and NaF 1 mM) supplied with protease inhibitor cocktails (Sigma-Aldrich, MO, USA). Protein concentration was measured using Bradford method. An equal amount of proteins (10 μg per well) was loaded on 12% SDS-PAGE gel and transferred onto PVDF membranes (EMD, Millipore). Blocking was carried out in 5% skim milk for 1 h at room temperature. After washing with PBS-T buffer, they were coincubated with anti-IL-1β (1 : 1000; Santa Cruz, USA), anti-IL-18 (1 : 1000; Elabscience, USA), anti-NLRP3 (1 : 500; Abcam, UK), anti-caspase 1 (1 : 1000, Santa Cruz, USA), anti-NLRC4 (1 : 1000; Santa Cruz, USA), and anti-β-actin (1 : 300; Santa Cruz, USA). Afterward, membranes were coincubated with secondary antibody m-IgGκ (1 : 1000; Santa Cruz, USA). Bound antibodies were developed using an ECL kit (Thermo Scientific; USA) and

![Figure 1](image_url)  
**Figure 1**: Schematic presentation of the experimental protocol. The cells in blank group were treated only with ATP (5 mM, 30 min). The cells in LPS control group were treated with LPS (10 μg/ml, 6 h) and ATP (5 mM, 30 min). In RT group, the cells that had been treated with 10 μM AEA for 24 h induced with LPS (10 μg/ml, 6 h), and in OT group, the cells were treated with AEA (10 μM, 24 h) after treatment with LPS (10 μg/ml, 6 h), and the last two groups were treated with ATP (5 mM, 30 min) after LPS induction.

| Gene name | Sequence (5′-3′) | Length (bp) |
|-----------|------------------|-------------|
| B2M       | Forward: CGCTACTCTCTCTCTCTCTGG | 143         |
|           | Reverse: GTCAACTTCAAATGTCGGATGGAT |            |
| NLRP3     | Forward: ATCTCCTTGTCTCTCACCA | 142         |
|           | Reverse: AGCTGACCAACCCAGAGCTTC |            |
| NLRC4     | Forward: GAACTGATCGACAGAATGAACG | 133         |
|           | Reverse: ACCCAAGCGGGCCGAGCTTTAT |          |
| ASC       | Forward: ACGAATCTCCTGGACCACC | 123         |
|           | Reverse: CTCGTATCCCCATGTGTCGAAG |            |
| IL-1β     | Forward: GATTCTGACTGATGAGATAATGC | 184         |
|           | Reverse: CAGAGGTACATTTCTCCCTAAC |          |
| IL-18     | Forward: GATTCTGACTGATGAGATAATGC | 166         |
|           | Reverse: CAGAGGTACATTTCTCCCTAAC |          |
2.8 Measurement of Caspase 1 Activity. The activity of caspase 1 was determined in cell lysate using Ac-WVAD-pNA (acetyl-Trp-Val-Ala-Asp-p-nitroanilide) (Sigma-Aldrich, MO, USA) as a chromogenic substrate according to the manufacturer’s protocol. In brief, the cells were lysed by resuspending in the chilled lysis buffer (100 mM Bicine, 1% Triton X-100, 250 mM KCl, 1 mM EDTA, 1 mM PMSF, 1 mM 4-(2-aminoethylbenzene) sulfonyl fluoride, 1 mM dithiothreitol (DTT), 1% NP-40, and pH 8.0). Insoluble materials were pelleted by centrifugation at 10000 RPM for 15 min at 4 °C, and the supernatant was isolated. Caspase 1 activity was measured by mixing 10 μl (100 μg protein) of extract and 90 μl of reaction buffer and 5 μl of 4 mM colorimetric substrate, then incubated at 37 °C. The release of pNA (p-nitroaniline) was monitored at 405 nm. The comparison of the absorbance of the substrate from a treated sample with an untreated control sample allowed the determination of fold change in caspase 1 activity.

2.9 IL-1β Secretion Assay. IL-1β concentration was measured in the medium of cultured cells using a specific sandwich-based ELISA kit according to the manufacturer’s instructions (Karmania Pars Gen, Kerman, Iran).

2.10 Statistical Analysis. All experiments reported in the present study were performed at least in triplicate. The data are reported as mean ± standard error of the mean (SEM). The statistical significance of the differences between groups was compared using the Student’s t-test and one-way analysis of variance (ANOVA), and subsequent post hoc analysis was performed using Tukey’s test. Differences with \( p < 0.05 \) were considered statistically significant.

3. Results

3.1 AEA Decreased Cell Viability Dose-Dependently. AGS cells were treated with LPS and/or AEA for 12 and 24 h, and the viability of cells was assayed using MTT assay. As exhibited in Figure 2, AEA showed a dose-dependent inhibitory effect on LPS-stimulated AGS cells (\( p < 0.05 \)). The viability of AGS cells had no significant differences between 12 and 24 h exposure times, and therefore, the subsequent experiments were performed using 10 μM AEA for 24 h.

3.2 AEA Suppressed Inflammasome-Related Genes in LPS-Stimulated Cells. The expression of all examined inflammasome-related genes was upregulated following LPS treatment. Among NLR family members, NLRP3 showed the highest upregulation (23.58 ± 2.24-fold). The expression of other inflammasome-related genes including NLRC4 (18.35 ± 1.7-fold), IL-1β (10.05 ± 1.21-fold), IL-18 (4.16 ± 0.81-fold), and ASC (4.78 ± 1.04-fold) was increased in LPS-stimulated AGS cells (\( p < 0.05 \)) (Figure 3). As shown in Figure 3, the treatment of AGS cells with AEA in both RT and OT experimental groups attenuated the elevated expression of all studied inflammasome genes (\( p < 0.05 \)). Treatment of AGS cells with AEA in the RT group had more reducing effects on the expression of NLRP3, IL-1β, and IL-18 genes when compared with the OT group (\( p < 0.05 \)). There were no significant differences in the expression of NLRC4 and ASC genes between RT and OT experimental groups.

3.3 AEA Suppressed Inflammasome Activation. The secretion of IL-1β and the activity of caspase 1 were determined in the medium or cell lysate of LPS-stimulated cells. The results revealed that the secretion of IL-1β and the activity of caspase 1 were increased after stimulation of AGS cells by 10 μg/ml LPS (\( p < 0.01 \); \( p < 0.05 \)) (Figure 4). Additionally, as shown in Figure 4, treatment of AGS cells with AEA significantly reduced IL-1β secretion in the RT group (\( p < 0.05 \)) and caspase 1 activity (\( p < 0.01 \); \( p < 0.05 \)) in both experimental groups. AEA in RT group had more reducing effects on IL-1β secretion (\( p < 0.05 \)) and caspase 1 activity (\( p < 0.001 \)) compared with OT group.

3.4 CB1 and TRPV1 Receptors Blockade Reversed AEA-Mediated Inflammasome Inhibition. The effective concentrations of two CBR antagonists were selected based on IL-1β secretion in the culture media using ELISA. AM-251 reversed the AEA effect on IL-1β secretion dose-dependently, and hence, the highest concentration of AM-251 was chosen for the next experiments (Figure 5(a)). Moreover, administration of 1 μM AMG-9810 led to a significant elevation in IL-1β secretion (Figure 5(a)). Accordingly, a concentration of 1 μM of AMG-9810 and AM-251 was selected for subsequent experiments.

Effect of CB1R and TRPV1 blockade using AM-251 and AMG-9810 on mRNA relative expression (Figure 5(b)) and protein expression (Figure 5(c)) of inflammasome-related genes in AEA-treated AGS cells is shown in Figure 5.
results showed that AM-251 reversed AEA action on NLRP3, NLRC4, and IL-1β (p < 0.05) in LPS-stimulated cells, which indicated that AEA regulated the expression of these genes via CB1 receptor. Although, ASC showed an upward trend after AM-251 treatment, the observed change was not significant. AMG-9810 administration inhibited AEA influence on IL-18 and IL-1β (p < 0.05) expression in LPS-stimulated cells, while other tested genes demonstrated no significant changes in the presence of AMG-9810 (Figure 5(b)).

As illustrated in Figure 6, measurement of cleaved IL-1β (Figure 6(a)) and the amount of secreted IL-1β in the medium (Figure 6(b)) showed that AM-251 and AMG-9810 could significantly reverse the inhibitory effects of AEA (p < 0.01; p < 0.05). While the expression of pro-caspase 1 was not altered, the expression of the cleaved caspase 1 (Figure 6(c)) and activity of caspase 1 (Figure 6(d)) was boosted after the application of antagonists in LPS-stimulated cells (p < 0.05; p < 0.01), indicated that AEA regulated these alterations via CB1R and TRPV1R.

4. Discussion

In the present study, we confirmed that the transcription levels of inflammasome component genes, including NLRP3, NLRC4, ASC, IL-1β, and IL-18, were increased in LPS-stimulated AGS cells, and AEA significantly diminished the expression of examined genes. This inhibitory effect was observed when LPS-stimulated inflammation was induced before or after treatment of AGS cells with AEA. It was observed that this endogenous cannabinoid reduced LPS-enhanced caspase 1 activity and IL-1β production and secretion in AGS cells. However, the application of AEA before LPS-mediated inflammation showed more profound effects on the downregulation of inflammasome-related genes and IL-1β secretion. Furthermore, it was found that the administration of selective CB1R antagonist (AM-251) attenuated AEA-induced downregulation of NLRP3 and NLRC4. Though AM-251 had no significant effect on the expression of IL-18 gene, AMG-9810 elevated its expression via blocking TRPV1R. IL-1β secretion was regulated via both CB1R and TRPV1R. Taken together, our data suggested that CB1R and TRPV1R may contribute to inhibitory effects of AEA on inflammasome activation and secretion of IL-1β in inflammatory AGS cells.
Recent studies suggest that inflammasome disorders and abnormal activation of IL-1β are involved in the generation and development of different chronic inflammatory diseases, such as lung cancer [22], diabetic cardiomyopathy [23], and gastric carcinogenesis [24]. Gastric cancer is known as one of the most common and deadly malignancies globally. Over the past decade, many lines of evidence highlighted the role of inflammasome activation and IL-1β hypersecretion on the occurrence and development of GC. Inflammasomes induced such effects mainly through blocking gastric acid secretion, alterations in epigenetics, assisting angiogenesis, and development of different chronic inflammatory diseases. D’Argenio and colleagues reported that TNBS-induced colitis was inhibited by AEA treatment [31]. Δ⁹-THC inhibited the secretion of IL-1β in LPS-stimulated human osteosarcoma cell line MG-63 via CB2R [32]. CBD suppressed the inflammatory effects of LPS-nigericin in THP-1 monocytes via inhibition of NLRP3 inflammasome activation [33]. In line with our observations, inhibitory effects of AEA pretreatment on CCL2 expression in human vascular smooth muscle cells [34], LPS-induced NO release in rat microglial [35], and production of TNF-α and IL-12 p40 in human keratinocytes [36] have been demonstrated. Moreover, the beneficial effects of AEA in various inflammatory disease models, such as LPS-induced pulmonary inflammation [37], liver damage [38], LPS-induced neuroinflammation [35], experimental periodontitis [39], intestinal inflammation [40, 41], colonic inflammation [42], and LPS-stimulated changes in circulating cytokines in rat [43], have been reported. Our findings are consistent with previous studies in the literature which suggested endocannabinoids via cannabinoid receptors reduced inflammation and proinflammatory cytokines.

To the best of our knowledge, the present results strongly show the first experimental evidence regarding the inhibitory activity of AEA on LPS-induced inflammasome activation and IL-1β secretion in AGS gastric cancer cells.

There are limited studies regarding the effects of AEA in gastrointestinal inflammatory diseases. D’Argenio and colleagues reported that TNBS-induced colitis was inhibited by AEA treatment [44]. Inhibition of AEA degradation using
chemical agents against its degradative enzymes such as fatty acid amide hydrolase or using genetic knockout models improved in inflammation [45], and ameliorated anandamide mediated antitumorigenic effect in some cancer models [46]. It was reported that pretreatment with WIN55,212-2, a synthetic CB1 receptor agonist, inhibited the expansion of stress-induced gastric ulcers in rats [47]. The gastroprotective effect of AEA and reduction of IL-1β in serum have also been reported after exposure of gastric mucosa to AEA in gastric lesions induced by water immersion and restrain stress (WRS) [48]. Furthermore, AEA synergistically augmented paclitaxel-induced apoptosis in gastric cancer cell lines [18].

5. Conclusion

In conclusion, our findings showed that AEA potentially acted as an anti-inflammatory molecule in AGS cells by inhibition of expression or activation of inflammasome components and secretion of IL-1β via CB1R and TRPV1R. Collectively, our results provide more evidence for the therapeutic value of endocannabinoid-based therapeutics for the treatment of GC.

Data Availability

The data used to support the findings of this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Shahid Chamran University of Ahvaz Research Council (Grant No: 97/3/02/16670).

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit et al., “Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012,” International Journal of Cancer, vol. 136, no. 5, pp. E359–E386, 2015.

[2] N. A. Wright, R. Poulsom, G. Stamp et al., “Trefoil peptide gene expression in gastrointestinal epithelial cells in inflammatory bowel disease,” Gastroenterology, vol. 104, no. 1, pp. 12–20, 1993.
[3] M. B. Piazuelo, R. P. Riechelmann, K. T. Wilson, and H. M. S. Algood, “Resolution of gastric cancer-promoting inflammation: a novel strategy for anti-cancer therapy,” in Molecular Mechanisms of Inflammation: Induction, Resolution and Escape by Helicobacter pylori, Current Topics in Microbiology and Immunology, vol 421, S. Backert, Ed., Springer, Cham, 2019.

[4] D. Sharma and T.-D. Kanneganti, “The cell biology of inflammasomes: mechanisms of inflammasome activation and regulation,” The Journal of Cell Biology, vol. 213, no. 6, pp. 617–629, 2016.

[5] S. Yin, C. Lan, H. Pei, and Z. Zhu, “Expression of interleukin 1β in gastric cancer tissue and its effects on gastric cancer,” Oncotargets and Therapy, vol. 9, p. 31, 2016.

[6] M. C. Camargo, R. Mera, P. Correa et al., “Interleukin-1β and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis,” Cancer Epidemiology and Prevention Biomarkers, vol. 15, no. 9, pp. 1674–1687, 2006.

[7] W. H. Wang, J. Q. Huang, G. F. Zheng, S. K. Lam, J. Karlberg, and B. C.-Y. Wong, "Non-steroidal anti-inflammatory drug use and the risk of gastric cancer: a systematic review and meta-analysis," Journal of the National Cancer Institute, vol. 95, no. 23, pp. 1784–1791, 2003.

[8] Z. Zhang, F. Chen, and L. Shang, “Advances in antitumor effects of NSAIDs,” Cancer Management and Research, vol. 10, pp. 4631–4640, 2018.

[9] H. Ashtktorab, A. Soleimani, G. Singh et al., “Safron: the golden spice with therapeutic properties on digestive diseases,” Nutrients, vol. 11, no. 5, p. 943, 2019.

[10] Y. I. Mahmoud and E. A. A. El-Ghaffar, “Spirulina ameliorates aspirin-induced gastric ulcer in albino mice by alleviating oxidative stress and inflammation,” Biomedicine & Pharmacotherapy, vol. 109, pp. 314–321, 2019.

[11] V. Di Marzo, T. Bisogno, L. De Petrocellisz, D. Melck, and B. Martin, “Cannabinimimetic fatty acid derivatives: the anandamide family,” Current Medicinal Chemistry, vol. 6, no. 8, pp. 721–744, 1999.

[12] E. I. Canela, E. Moreno, V. Casado, M. Cavic, and A. Krivokuca, “The endocannabinoid system as a target in cancer diseases: are we there yet?,” Frontiers in Pharmacology, vol. 10, p. 339, 2019.

[13] A. Munson, L. Harris, M. Friedman, W. Dewey, and R. Carchman, “Antineoplastic activity of cannabinoids,” Journal of the National Cancer Institute, vol. 55, no. 3, pp. 597–602, 1975.

[14] A. Howlett, F. Barth, T. Bonner et al., “International Union of Pharmacology. XXVII. Classification of cannabinoid receptors,” Pharmacological Reviews, vol. 54, no. 2, pp. 161–202, 2002.

[15] R. G. Pertwee and R. Ross, “Cannabinoid receptors and their ligands,” Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA), vol. 66, no. 2-3, pp. 101–122, 2002.

[16] P. H. Reggio, “Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown,” Current Medicinal Chemistry, vol. 17, no. 14, pp. 1468–1486, 2010.

[17] P. M. Zygmont, J. Petersson, D. A. Andersson et al., “Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide,” Nature, vol. 400, no. 6743, pp. 452–457, 1999.

[18] H. Miyato, J. Kitayama, H. Yamashita et al., “Pharmacological synergism between cannabinoids and paxiltaex in gastric cancer cell lines,” Journal of Surgical Research, vol. 155, no. 1, pp. 40–47, 2009.

[19] C. Turcotte, F. Chouniard, J. S. Lefebvre, and N. Flamand, “Regulation of inflammation by cannabinoids, the endocannabinoids 2-arachidonoyl-glycerol and arachidonoyl-ethanolamide, and their metabolites,” Journal of Leukocyte Biology, vol. 97, no. 6, pp. 1049–1070, 2015.

[20] Y.-L. Hung, S.-C. Wang, K. Suzuki et al., “Bavachin attenuates LPS-induced inflammatory response and inhibits the activation of NLRP3 inflammasome in macrophages,” Phytomedicine, vol. 59, p. 152785, 2019.

[21] S. A. Bustin, V. Benes, J. A. Garson et al., “The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments,” Clinical Chemistry, vol. 55, no. 4, pp. 611–622, 2009.

[22] Y. Wang, H. Kong, X. Zeng et al., “Activation of NLRP3 inflammasome enhances the proliferation and migration of A549 lung cancer cells,” Oncology Reports, vol. 35, no. 4, pp. 2053–2064, 2016.

[23] X. Wang, J. Pan, H. Liu et al., “AIM2 gene silencing attenuates diabetic cardiomyopathy in type 2 diabetic rat model,” Life Sciences, vol. 221, pp. 249–258, 2019.

[24] S. Li, X. Liang, L. Ma et al., “MiR-22 sustains NLRP3 expression and attenuates _H. pylori_ -induced gastric carcinogenesis,” Oncogene, vol. 37, no. 7, pp. 884–896, 2018.

[25] F. Perri, F. Terracciano, M. Gentile, A. Merla, D. Scimeca, and A. Zullo, “Role of interleukin polymorphisms in gastric cancer:’pros and cons’,” World Journal of Gastrointestinal Oncology, vol. 2, no. 6, pp. 265–271, 2010.

[26] C. Persson, L. Engstrånd, O. Nyrén et al., “Interleukin 1-β gene polymorphisms and risk of gastric cancer in Sweden,” Scandinavian Journal of Gastroenterology, vol. 44, no. 3, pp. 339–345, 2009.

[27] V. Deswaerpe, P. Nguyen, A. West et al., “Inflammasome adaptor ASC suppresses apoptosis of gastric cancer cells by an IL18-mediated inflammation-independent mechanism,” Cancer Research, vol. 78, no. 5, pp. 1293–1307, 2018.

[28] S. Xu, X. Li, Y. Liu, Y. Xia, R. Chang, and C. Zhang, “Inflammasome inhibitors: promising therapeutic approaches against cancer,” Journal of Hematology & Oncology, vol. 12, no. 1, p. 64, 2019.

[29] E. Pagano and F. Borrelli, Targeting cannabinoid receptors in gastrointestinal cancers for therapeutic uses: current status and future perspectives, Taylor & Francis, 2017.

[30] A. Ortega, V. García-Hernández, E. Ruiz-Garcia et al., “Comparing the effects of endogenous and synthetic cannabinoid receptor agonists on survival of gastric cancer cells,” Life Sciences, vol. 165, pp. 56–62, 2016.

[31] S. Suryavanshi, I. Kovalchuk, and O. Kovalchuk, “Cannabinoids as key regulators of inflammasome signalling: a current perspective,” Frontiers in Immunology, vol. 11, p. 3638, 2020.

[32] L. Yang, F.-F. Li, Y.-C. Han, B. Jia, and Y. Ding, “Cannabinoid receptor CB2 is involved in tetrahydrocannabinol-induced anti-inflammation against lipopolysaccharide in MG-63 cells,” Mediators of Inflammation, vol. 2015, Article ID 362126, 11 pages, 2015.

[33] C. Liu, H. Ma, A. L. Slitt, and N. P. Seeram, “Inhibitory effect of cannabidiol on the activation of nlrp3 inflammasome is associated with its modulation of the p2x7 receptor in human monocytes,” Journal of Natural Products, vol. 83, no. 6, pp. 2025–2029, 2020.

[34] B. Pflüger-Müller, J. A. Oo, J. Heering et al., “The endocannabinoid anandamide has an anti-inflammatory effect on CCL2...
expression in vascular smooth muscle cells," Basic Research in Cardiology, vol. 115, no. 3, p. 34, 2020.

[35] N. Malek, K. Popiolek-Barczyk, J. Mika, B. Przewlocka, and K. Starowicz, "Anandamide, acting via CB2 receptors, alleviates LPS-induced neuroinflammation in rat primary microglial cultures," Neural Plasticity, vol. 2015, Article ID 130639, 10 pages, 2015.

[36] V. Chiurchiù, C. Rapino, E. Talamonti et al., "Anandamide, acting via CB2 receptors, alleviates LPS-induced neuroinflammation in rat primary microglial cultures," Neural Plasticity, vol. 2015, Article ID 130639, 10 pages, 2015.

[37] V. Chiurchiù, C. Rapino, E. Talamonti et al., "Anandamide suppresses proinflammatory T cell responses in vitro through type-1 cannabinoid receptor–mediated mTOR inhibition in human keratinocytes," The Journal of Immunology, vol. 197, no. 9, pp. 3545–3553, 2016.

[38] E. Berdyshev, E. Boichot, M. Corbel, N. Germain, and V. Lagente, "Effects of cannabinoid receptor ligands on LPS-induced pulmonary inflammation in mice," Life Sciences, vol. 63, no. 8, pp. PL125–PL129, 1998.

[39] E. Rettori, A. De Laurentiis, M. Z. Zubilete, V. Rettori, and J. C. Elverdin, "Anti-inflammatory effect of the endocannabinoid anandamide in experimental periodontitis and stress in the rat," Neuroimmunomodulation, vol. 19, no. 5, pp. 293–303, 2012.

[40] F. Massa, G. Marsicano, H. Hermann et al., "The endogenous cannabinoid system protects against colonic inflammation," The Journal of Clinical Investigation, vol. 113, no. 8, pp. 1202–1209, 2004.

[41] M. Izzo and M. Camilleri, "Cannabinoids in intestinal inflammation and cancer," Pharmacological Research, vol. 60, no. 2, pp. 117–125, 2009.

[42] M. A. Storr, C. M. Keenan, D. Emmerdinger et al., "Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB 1 and CB 2 receptors," Journal of Molecular Medicine, vol. 86, no. 8, pp. 925–936, 2008.

[43] M. Roche, J. P. Kelly, M. O’Driscoll, and D. P. Finn, "Augmentation of endogenous cannabinoid tone modulates lipopolysaccharide-induced alterations in circulating cytokine levels in rats," Immunology, vol. 125, no. 2, pp. 263–271, 2008.

[44] G. D’Argenio, M. Valenti, G. Scaglione, V. Cosenza, I. Sorrentini, and V. Di Marzo, "Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation," The FASEB Journal, vol. 20, no. 3, pp. 568–570, 2006.

[45] J. E. Schlosburg, S. G. Kinsey, and A. H. Lichtman, "Targeting fatty acid amide hydrolase (FAAH) to treat pain and inflammation," The AAPS Journal, vol. 11, no. 1, pp. 39–44, 2009.

[46] J. Ravi, A. Sneh, K. Shilo, M. W. Nasser, and R. K. Ganju, "FAAH inhibition enhances anandamide mediated antitumorigenic effects in non-small cell lung cancer by downregulating the EGF/EGFR pathway," Oncotarget, vol. 5, no. 9, pp. 2475–2486, 2014.

[47] M. P. Germanò, V. D’Angelo, M. R. Mondello et al., "Cannabinoid CB 1-mediated inhibition of stress-induced gastric ulcers in rats," Naunyn-Schmiedeberg’s Archives of Pharmacology, vol. 363, no. 2, pp. 241–244, 2001.

[48] A. Dembowski, Z. Warzecha, P. Ceranowicz et al., "Cannabinoids in acute gastric damage and pancreatitis," Journal of Physiology and Pharmacology, vol. 57, Suppl 5, pp. 137–154, 2006.