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IL-8 analogue CXCL8 (3-72) K11R/G31P, modulates LPS-induced inflammation via AKT1-NF-kβ and ERK1/2-AP-1 pathways in THP-1 monocytes

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ABSTRACT

IL-8 is elevated during inflammation, and it initiates cascade of down-stream reactions. Its antagonist, CXCL8 (3–72) K11R/G31P (G31P), represses inflammatory reactions via competitive binding to CXC chemokine family, preferentially G protein-couple receptors (GPCRs) CXCR1/2. This study reports the effect of G31P on the transcription profile of lipopolysaccharide (LPS) induced inflammation in THP-1 monocytes *ex-vivo*. LPS (1 μg/ml) induced elevation of IL-8 was significantly reduced by G31P (20 μg/ml and 30 μg/ml), with relatively increased inhibition of CXCR2 than CXCR1. Transcription of IL-1β, IL-6, and TNF-α were significantly inhibited, while IL-10 remained relatively unchanged. G31P treatment also had repressing effect on the inflammatory associated enzymes COX-2, MMP-2, and MMP-9. Significant restriction of c-Fos, and NF-kβ mRNA expression was observed, while that of c-Jun was marginally elevated. Conversely, SP-1 mRNA expression was seen to increase appreciably by G31P treatment. While the translation of pAKT, pERK1/2, and p65- NF-kβ were down-regulated by the G31P following THP-1 cells stimulation with LPS, reactive oxygen species (ROS) expression was on the positive trajectory. Collectively, the IL-8 analogue, G31P, modulates the inflammatory profile of LPS induced inflammation in THP-1 monocytes via AKT1-NF-kβ and ERK1/2-AP-1 pathways.

1. Introduction

Inflammation is inevitably a ubiquitous cellular and biochemical reaction required to maintain homeostasis during cellular or tissue insult in a biological system. Causes of inflammation are varied, encompassing both biological and non-biological agents, and its origin could be endogenous or exogenous. Inflammatory reactions are present in both innate and adaptive immune reactions processes, an indication of the importance of optimized inflammatory reactions for well-being [1,2]. Primarily, inflammation is meant to arrest and confine the causative agents' turbulence, get rid of compromised cells or tissues, and safeguard the body from systemic damage. However, inflammatory reactions can be exaggerated and may result in self-damage as in the case of auto-immune diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis (RA), multiple sclerosis (MS), type 1 diabetes mellitus (T1DM), systemic lupus erythematous (SLE), psoriasis, and scleroderma [3,4].

Following tissue or cellular insults, the innate immune response communicates the invading threat to immune cells via signaling molecules called chemokines or cytokines. Invading pathogens express genetically conserved pathogen-associated molecular patterns (PAMPs) [5]. These small molecular motifs harbored by pathogens are ligands to germ-line encoded transmembrane receptors displayed by the innate immune cells, collectively referred to as pattern-recognition receptors (PRRs). Well characterized PRRs include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs),

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retinoic acid-inducible gene-I- (RIG-I-) like receptors (RLRs), and C-type lectin receptors (CLRs) [6,7]. However, TLRs and NLRs are the major types of PRRs in pathogen associated inflammation. TLRs recognize PAMPS while in the case of sterile or none infectious inflammation, danger/damage-associated molecular patterns (DAMPs), also referred to as alarmin, are sensed by intracellular NLRs, and subsequently signal the presence of cellular damage [8,9].

The binding of PAMPs and DAMPs to their cognate PRRs, initiates cascades of inflammatory reactions downstream. In an undisturbed host, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) is made inactive by clinging to NF-κβ inhibitor (Iκβ). However, when TLRs get activated by PAMPs, Iκβ disengages NF-κβ and it translocate into the nucleus [10,11]. Other transcription factors such as activator protein-1 (AP-1) and interferon regulatory factors (IRFs) are also activated following PAMPs/DAMPs-ligand complex. The translation of these inflammatory related gene yields pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IL-10, and TNF-α [12,13].

The mechanism of actions of existing anti-inflammatory drugs function by interrupting various inflammatory pathways to minimize or block the secretion of pro-inflammatory associated proteins. Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, sulindac, indomethacin, ibuprofen, diclofenac, and celecoxib function via inhibition of either or both cyclooxygenase 1 and 2 (COX-1 and COX-2) [14–16]. Available steroidal anti-inflammatory drugs such as corticosteroids confer immuno-suppressor effect on users, and protracted use predisposes patients to osteoporosis and fracture. Anti-tumor necrosis factor alpha (TNF-α) and anti- $\alpha_4\beta_7$ integrin are biologics employed clinically to counter inflammatory activity in some diseases. These agents have also been reported as risk for lymphomas, restricting their use [17–19]. The risks associated with most of the available anti-inflammatory agents give room to further explore for effective but less side effects or risk free drugs.

CXCL8 antagonist, CXCL8 (3-72) K11R/G31P (G31P), has been reported as neutrophils and macrophages inhibitor. This chemokine analogue is a ligand to G protein-couple receptors (GPCRs) CXCR1 and CXCR2 [20]. These receptors are expressed on membranes of polymorphonuclear cells (PMNs), monocytes, astrocytes, eosinophil, endothelia cells and mast cells [21,22]. They have been implicated in many diseases, and competitively annulling their reception to their cognate ligand is seen as potential therapeutic target. The therapeutic potential of G31P as a competitive binder to CXCR1/2 to inhibit downstream signaling of IL-8 pathway has been reported in previous studies [23,24]. Its efficacy against breast, liver, lung, ovarian, and prostate tumors has been emphasized, and more recently its potential application against inflammatory bowel diseases, particularly ulcerative colitis, has been underscored [25–27]. However, the effect of G31P on transcription profile of inflammatory reactions *in vitro* remains unclear. This study reports that G31P exhibits varied effects on the transcriptional profiles of lipopolysaccharide (LPS) induced inflammation in THP-1 monocytes *ex-vivo*.

2. Materials and methods

2.1. Chemicals and Reagents

The synthesis of G31P was as described previously [20]. The cell line, Human acute monocytic leukemia cell line THP-1 (from American Type Culture Collection (ATCC), USA) was used for all the experiment. Reagents and media used include; Dojindo Cell Counting Kit-8 (CCK-8), Japan; Fetal bovine serum (FBS), PAA, Australia; Rowell Park Memorial Institute Medium-1640 (RPMI-1640), allBio Life Sciences, China; 50 units/mL of penicillin and streptomycin, Transgen Biotech, China, Lipopolysaccharide (LPS) from *Escherichia coli*, Phosphate buffer saline (PBS), and TAE, all from solarbio, China. Antibodies used were anti-p-ERK1/2 (1:500), anti-ERK1/2 (1:500), anti-p65 NF-kB (1:500), and β-actin (1:500), all from proteintech, China. Kits for Enzyme-linked immuno-sorbent assay (ELISA), were from Lengton Bioscience Co. Ltd., China.

2.2. Cell culture

THP-1 cell culture was done in RPMI with 10% FBS, 50 IU/ml and 50 μg/ml penicillin and streptomycin respectively, with/without 50 nM of 2-mecaptoethanol (2ME). Culture incubation conditions were set at 37 °C in a humidified atmosphere with 5% CO_2. Cells were harvested by centrifugation at 1000 rpm for 5 min. Estimated cells population for various assays ranged from 3×10^3 to 1×10^6 cells per well. Medium for cells was changed after every other day.

2.3. Cell viability assay

Employing CCK-8 assay technique, the effect of G31P on THP-1 cells viability was assessed. Cells suspending in complete medium without 2ME, and treated with varied concentrations of G31P or G31P + LPS were seeded in triplicates at a density of 3×10^3 cells per 100 μl per well in 96 well-plates. The plates were incubated for 24 h and 48 h, and 10 μl of CCK-8 reagent added to each well. After additional hour of incubation, the absorbance of the cultures were measure with plate-reader (Thermo Electron Corporation, Finland) at a wavelength of 450 nm. The estimation of cell viability was calculated by the formula: (Mean absorbance of control – Mean absorbance of treatment)/Mean absorbance of control.

2.4. RNA extraction and cDNA amplification

Estimated 1×10^6 THP-1 cells in complete medium without 2ME were dispensed into six-well plate, and treated with LPS, and LPS with G31P. The cells were incubated for 24 h and then harvested by centrifugation at 1000 rpm for 5 min. Total RNA was extracted from sediment using RNAiso Plus (Takara Bio Inc). Following RNA concentration quantification (Biorad nanodrop, USA), complementary DNA (cDNA) was synthesized with kits from Transgen Biotech, China. The cDNA were subjected to PCR to amplify target genes. All primers were obtained from Invitrogen Biotechnology Co (Dalian). Primer sequences are available in Table 1. GAPDH was used as reference gene. The PCR products were subjected to 2% agarose gel electrophoresis, and bands detected with Tanon 1600 Gel Image System, Shanghai, China. Bands intensity were calculated by semi-quantitative method using ImageJ software, and presented as means of independent triplicate experiments relative to GAPDH.

2.5. ELISA assay

Using Enzyme-linked immuno-sorbent assay (ELISA) kit from Lengton Bioscience Co. Ltd., China, culture supernatant of the various treatments were analyzed for IL-8 concentrations. Samples preparation were guided by the dictates of the product's insert. Absorbance were estimated with plate reader (Thermo Electron Corporation, Finland), and presented as means of absorbance from three repeated experiments.

2.6. Reactive oxygen species (ROS) assay

THP-1 cells were seeded at a density of 10^5 in six well plates, and treated with indicated concentrations of G31P. The cells where incubated for 3 h in a humidified atmosphere at 37 °C and 5% CO_2. The cultures were then stimulated with 1 μg/ml LPS, and incubated for additional hour. After the stipulated time, the cultures were harvested, washed twice with PBS, suspended in 10 μM of H2DCFDA in serum free medium, and incubated under same conditions as above for 30 min. After washing twice with PBS, fluorescence signaling was measured and analyzed by flow cytometry (Accuri C6, BD Bioscience, San Jose, CA,

Table 1
Characteristics of primers used in the study.

Accession number	Name	Product length(bp)	Sequence	Length	Tm	%GC
NM_000576.2	Human IL-1B F	124	GTGGCAATGAGGATGACTTGTTC	23	60.12	47.83
	Human IL-1B R		TAGTGGTGGTCGGAGATTCGTA	22	60.36	50.00
NM_000600.3	Human IL-6F	118	AGCCACTCACCTCTTCAGAAC	21	59.65	52.38
	Human IL-6 R		GCCTCTTTGCTGCTTTCACAC	21	60.6	52.38
NM_000572.2	Human IL-10F	138	GTGATGCCCCAAGCTGAGA	19	59.7	57.89
	Human IL-10 R		CACGGCCTTGCTCTTGTTTT	20	59.61	50.00
NM_000594.2	Human TNF-a F	93	CTGCTGCACTTTGGAGTGAT	20	58.47	50.00
	Human TNF-a R		AGATGATCTGACTGCCTGGG	20	58.87	55.00
NM_000625.3	Human iNOS (2A) F	118	CATCCTCTTTGCGACAGAGAC	21	58.73	52.38
	Human iNOS (2A) R		GCAGCTCAGCCTGTACTTATC	21	58.52	52.38
NM_000963.2	Human COX 2F	128	CAGCACTTCACGCATCAGTT	20	59.13	50.00
	Human COX 2 R		CGCAGTTTACGCTGTCTAGC	20	59.36	55.00
NM_003998.2	Human NF-Kb F	103	TGAGTCCTGCTCCTTCCA	18	57.02	55.56
	Human NF-Kb R		GCTTCGGTGTAGCCCATT	18	57.36	55.56
NM_138473.2	Human SP-1F	130	GGTGCCTTTTCACAGGCTC	19	59.04	57.89
	Human SP-1 R		CATTGGGTGACTCAATTCTGCT	22	59.04	57.89
NM_002228.3	Human c-Jun F	242	TGGAAACGACCTTCTATGACGA	22	59.18	45.45
	Human c-Jun R		GTTGCTGGACTGGATTATCAGG	22	58.79	50.00
NM_005252.3	Human c-Fos F	280	GGATAGCCTCTCTTACTACCAC	22	56.88	50.00
	Human c-Fos R		TCCTGTCATGGTCTTCACAACG	22	60.55	50.00
NM_002046.3	Human GAPDH F	87	TGCACCACCAACTGCTTAGC	20	61.17	55.00
	Human GAPDH R		GGCATGGACTGTGGTCATGAG	21	61.17	55.00
NM_000634.2	Human CXCR1 F	200	GAGCCCCGAATCTGACATTA	20	57.09	50.00
	Human CXCR1 R		GCAGACACTGCAACACACCT	20	61.1	55.00
NM_001557.3	Human CXCR2 F	202	ATTCTGGGCATCCTTCACAG	20	57.57	50.00
	Human CXCR2 R		TGCACTTAGGCAGGAGGTCT	20	60.55	55.00

USA).

2.7. Protein extraction and western blotting

Cells were collected by centrifugation and their total protein harvested with protein extraction cocktail from KeyGen BioTEC, China. 50 µl of the ice-cold cocktail consisting of lysis buffer, protease and phosphatase inhibitors was added to the cell sediment on ice, and homogenized for 10 s every 10 min for 40 min. This was followed by centrifugation at 12000*g* for 5 min, and the supernatant collected as the total protein. KeyGen BioTEC protein assay kit was used to estimate the protein concentration, and equal concentrations were loaded for SDS-PAGE. After running SDS-PAGE, the proteins were blotted with 0.45 µm thick polyvinylidene difluoride (PVDF) membrane, and blocked for 2 h with 5% skimmed milk (BD, Difco, USA). The membrane was Tris-Buffered Saline with Tween 20 (TBST) washed trice, each time for 15 min. The membranes were incubated at 4 °C for 18 h in primary antibodies as indicated above. After three times wash with TBST, the membranes were incubated at room temperature for 1 h in HRP conjugated anti-rabbit secondary antibody. Enhanced-chemi-luminescence (ECL) reagent (KeyGen BioTEC) was applied, and bands detected with ChemiDoc MP imaging system (Biorad, USA). Bands quantification was done with Image-Pro Plus version 6.0 software (Microsoft Media Cybernetics, Bethesda, MD, USA).

2.8. Statistical analysis

Data obtained were analyzed with GraphPad Prism 5.0, and presented as mean ± SEM. We explored differences among the treatment group with one way analysis of variance (ANOVA) and Turkey's post hoc analysis was used for comparison. Statistical significance was pegged at p-value < 0.05.

3. Results

3.1. G31P restricts THP-1 monocytes viability, and IL-8-CXCR1/2 expressions

To assess the effect of G31P on THP-1 cells viability, CCK-8 assay was performed. It was observed that significant inhibition occurred at concentrations of 20 µg/ml and 30 µg/ml after 48 h incubation if cells are treated with only G31P. Co-treated with LPS (1 µg/ml) and G31P exhibited significant inhibition at 30 µg/ml after 48 h incubation. In both treatments, no significant variation was observed after 24 h incubation. The mRNA expression of IL-8 receptors CXCR1/2 were estimated after 24 h following induction of inflammation by LPS, and G31P treatment. LPS stimulation marginally elevated CXCR1 mRNA expression compared to Control group, but this was reverted by G31P treatment. Rather, a significant increase in CXCR2 mRNA was seen in LPS stimulated cells compared to Control, and similarly, was mitigated by G31P. Both transcription and translation levels of IL-8 were determined by RT-PCR and ELISA techniques respectively, and significant down-regulation of IL-8 mRNA and protein was noted (Fig. 1).

3.2. G31P treatments down-regulate inflammatory cytokines expression in LPS induced inflammation of THP-1 monocytes

The transcriptional activity of selected inflammatory cytokines where measured by RT-PCR. While LPS significantly induced TNF-α mRNA expression, marginal elevation in that of IL-1β and IL-6 were noted. These were however significantly down-regulated by G31P in concentration dependent manner. IL-10 mRNA expression on the other hand, remained relatively unchanged compared with Control and LPS induced groups (Fig. 2).

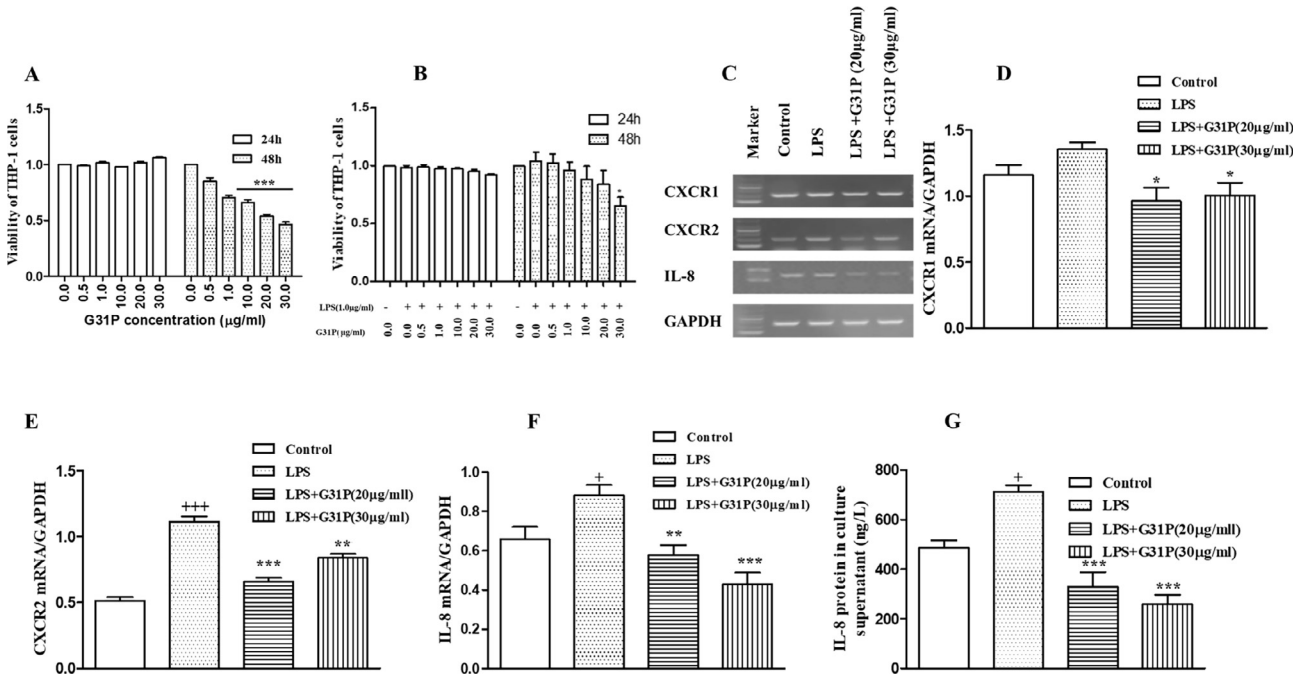

Fig. 1. Inhibition of THP-1 cells viability, IL-8, and CXCR1/2 receptors expressions. A. THP-1 cells treated with only G31P (mean absorbance measured at 450 nm). B. THP-1 cells induced with LPS and treated with G31P (mean absorbance measured at 450 nm). C. Representative RT-PCR band. D-F. Relative expression of CXCR1, CXCR2, and IL-8 mRNAs, with GAPDH as internal reference. G. IL-8 protein levels. Data are presented as mean ± SEM of independent triplicate experiments. $^{+++}p < 0.001$, $^{++}p < 0.01$, $^{+}p < 0.05$ vs Control group. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs LPS induced group.

3.3. G31P treatment down-regulates mRNA expression of inflammatory associated enzymes in LPS induced THP-1 cells

The mRNA expression of inflammatory associated enzymes including COX-2, MMP-2, and MMP-9 were assessed. Relatively higher concentration of G31P (30 μg/ml) was required to caused significant inhibition of COX-2 mRNA, while both treatment concentrations reverted LPS induced elevation of MMP-2 and MMP-9. On the contrary, iNOS mRNA were undetectable (results not shown) in all the experimental groups (Fig. 3).

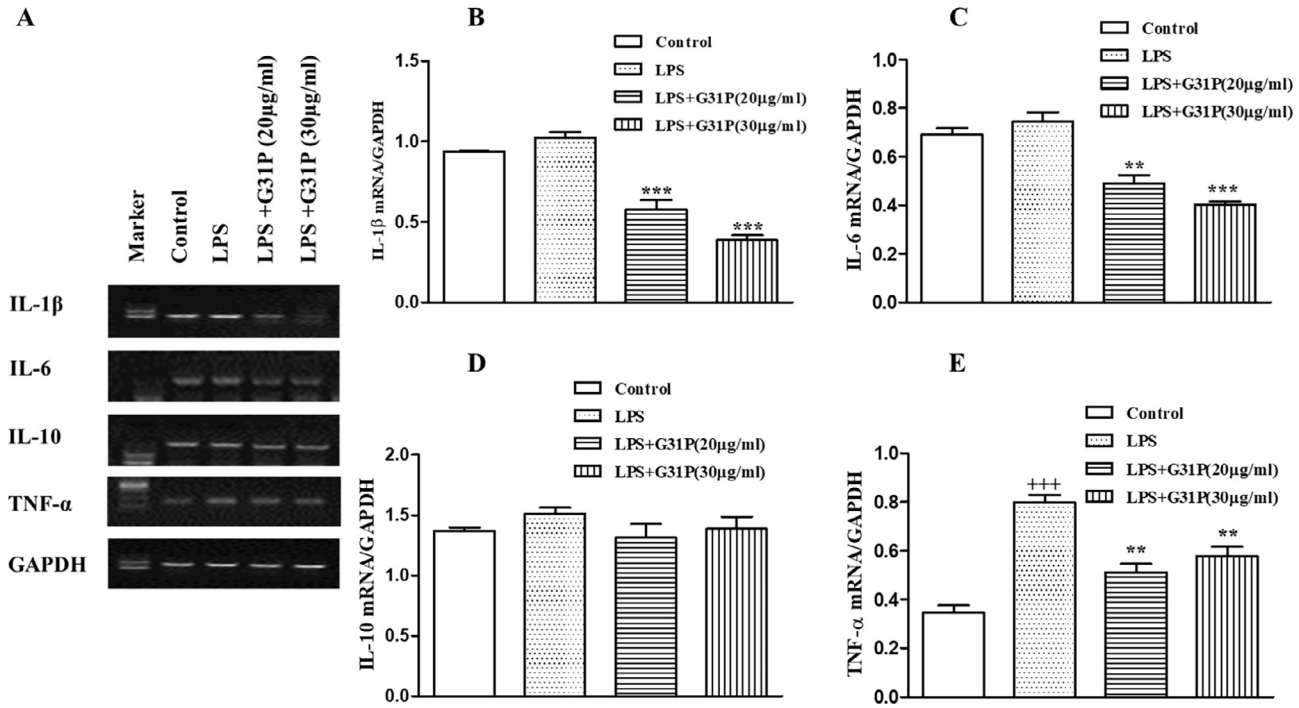

Fig. 2. G31P inhibition of inflammatory cytokines. A. Representative RT-PCR band. B-E. Relative expression of IL-1β, IL-6, IL-10, and TNF-α mRNAs respectively, with GAPDH as internal reference. Results are presented as mean ± SEM of independent triplicate experiments. $^{+++}p < 0.001$ vs Control. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs LPS induced group.

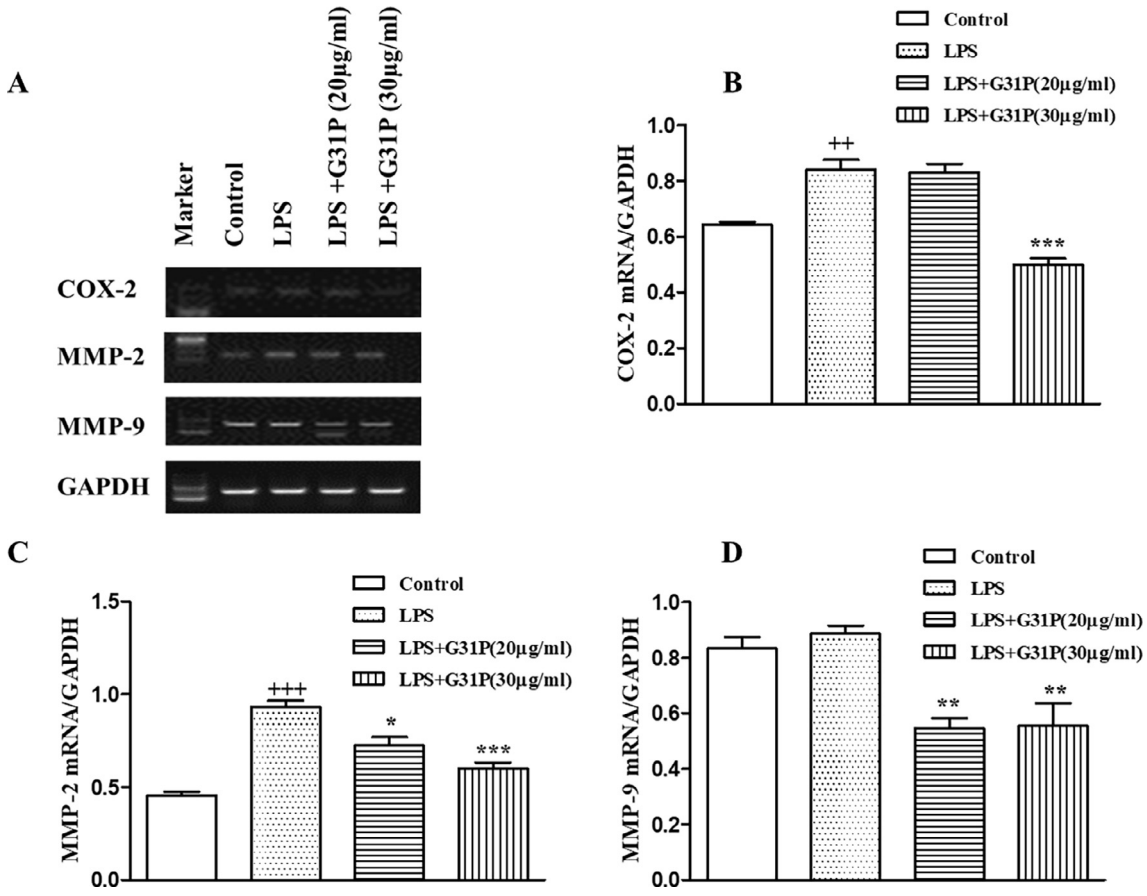

Fig. 3. Inhibition of inflammatory associated enzymes by G31P. A. Representative RT-PCR band. B-D. Relative expression of COX-2, MMP-2, and MMP-9 mRNAs respectively, with GAPDH as internal reference. All results are presented as mean ± SEM of independent triplicate experiments. $^{+++}p < 0.001$, $^{++}p < 0.01$ vs Control. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs LPS induced group.

3.4. G31P regulates inflammatory associated transcription factors

To further probe how G31P affects cytokines expression, inflammatory associate transcription factors including c-Fos, c-Jun, SP-1, and NF-kβ were detected by RT-PCR. Generally, no significant difference was observed between the Control and LPS group. However, G31P treatment significantly retards mRNA expression of c-Fos and NF-kβ, while marginal elevation of c-Jun mRNA was observed. Also, SP-1 mRNA was seen to increase appreciably by G31P treatment in concentration dependent manner (Fig. 4).

3.5. G31P regulates inflammatory cytokines expression via AKT1, ERK1/2, and ROS pathways

Employing western blot techniques, the levels of pERK1/2, pAKT1, and p65-NF-kβ were determined. Significant pAKT1 down-regulation following G31P treatment was accompanied with diminishing p65-NF-kβ, thereby restricting its transcriptional capability. This observation confirms the significant inhibition of NF-kβ mRNA as indicated in Fig. 4. Additionally, pERK1/2 expression was suppressed by G31P treatment compared with Control and LPS induced groups. However, the downstream effect of this hindrance was significant on the AP-1 protein subunit c-Fos mRNA, but not c-Jun. Flow cytometry analysis revealed that G31P increases ROS levels of LPS induced THP-1 monocytes in concentration-wise (Fig. 5).

4. Discussion

It has been demonstrated in the present study that G31P exhibits significant cellular inhibition after 48 h culture, a possible explanation to why previous studies administered it every other day in *in vivo* experiments. The membrane bound G31P receptors, CXCR1/2 are variably increased in expression during inflammation. However, enhanced expression of CXCR2 is seen during dys-homeostasis compared with CXCR1. These observations commensurate with previous *in vivo* and *in vitro* studies [28]. The main target of G31P, IL-8, which plays a crucial role during cellular or tissue disturbance was significantly elevated at both the transcription and translation levels by LPS. IL-8 has been established as a key promoter of inflammatory associated diseases including IBD, RA, and various forms of cancer [29], and its optimum regulation is appreciated in such diseases. In concentration-wise, G31P demonstrated appreciable down-regulation of IL-8 expression, suggesting the agent mitigates IL-8 expression in inflammation.

Other inflammatory cytokines which showed downward expression by G31P are IL-1β, IL-6, and TNF-α. These cytokines are generally elevated during inflammation, and their sustenance has been linked to poor prognosis of several diseases. In addition, the continuous elevation of these cytokines have been associated with cancer initiation and progression. IL-6 for instance is a cancer promoter, and its over-expression goes with poor clinical outcomes. However, its expression can be beneficial during acute inflammation as it contributes to the onset of innate immune response, via its ability to induce C-reactive protein and serum amyloid A during acute response to cellular insult [30]. Its activation has influence on T cells differentiation, particularly Treg and Th17 cells. IL-6 has been reported to promote colorectal cancer via IL-17A up-regulation [30]. On the other hand, Treg up-regulation confers tumor-suppressing activity, but other studies have indicated Treg could enhance breast cancer [31–33]. Previous *in vivo* studies revealed G31P

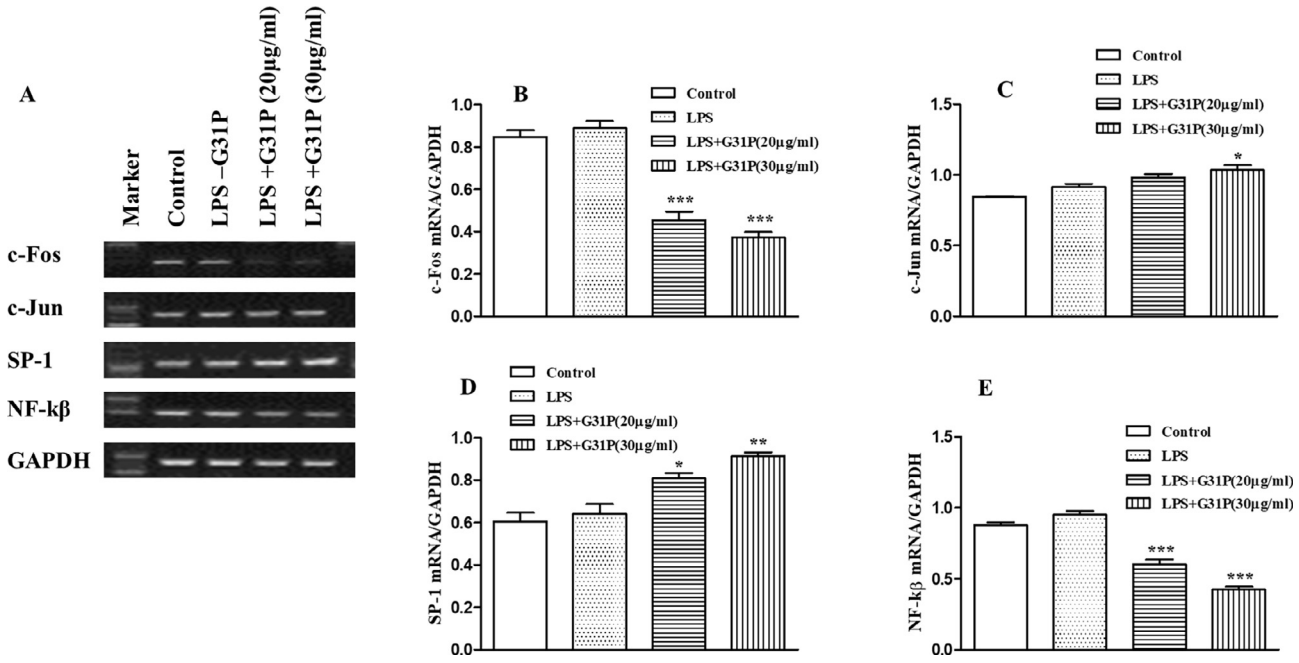

Fig. 4. Regulation of inflammatory associated transcription factors by G31P. A. Representative RT-PCR bands. B-E. Relative mRNA expressions of c-Fos, c-Jun, SP-1, and NF-kβ respectively, with GAPDH as internal reference. Results are shown as mean ± SEM of independent triplicate experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs LPS induced group.

selectively inhibits IL-17A, but not IL-17F. This observation will require further study focusing on the T cells family [26]. In IBDs, IL-6 seems to function to restrict T cell induced apoptosis, and facilitate the progression of the disease to cancer [34]. Also, IL-6 signaling in endothelial cells invites white blood cells to the inflamed region, serving as leukocyte recruiting agent. However, G31P mitigation of IL-6 expression by THP-1 monocytes during inflammation reveals its potential in combating the possible exaggerated effect of IL-6.

IL-1β and TNF-α like other cytokines are pluripotent in function. However, their continuous secretion correlates with disease severity, an indication that their persistent elevation contribute to chronic inflammatory diseases such as ulcerative colitis, Crohn's disease, and rheumatoid arthritis, and also confer implications on cancer initiation and progression [35]. IL-1β is a well-known pro-inflammatory marker, and plays a regulatory role in dys-homeostasis. It is a key monocyte, macrophage, and neutrophils activator, and signals for the presence of

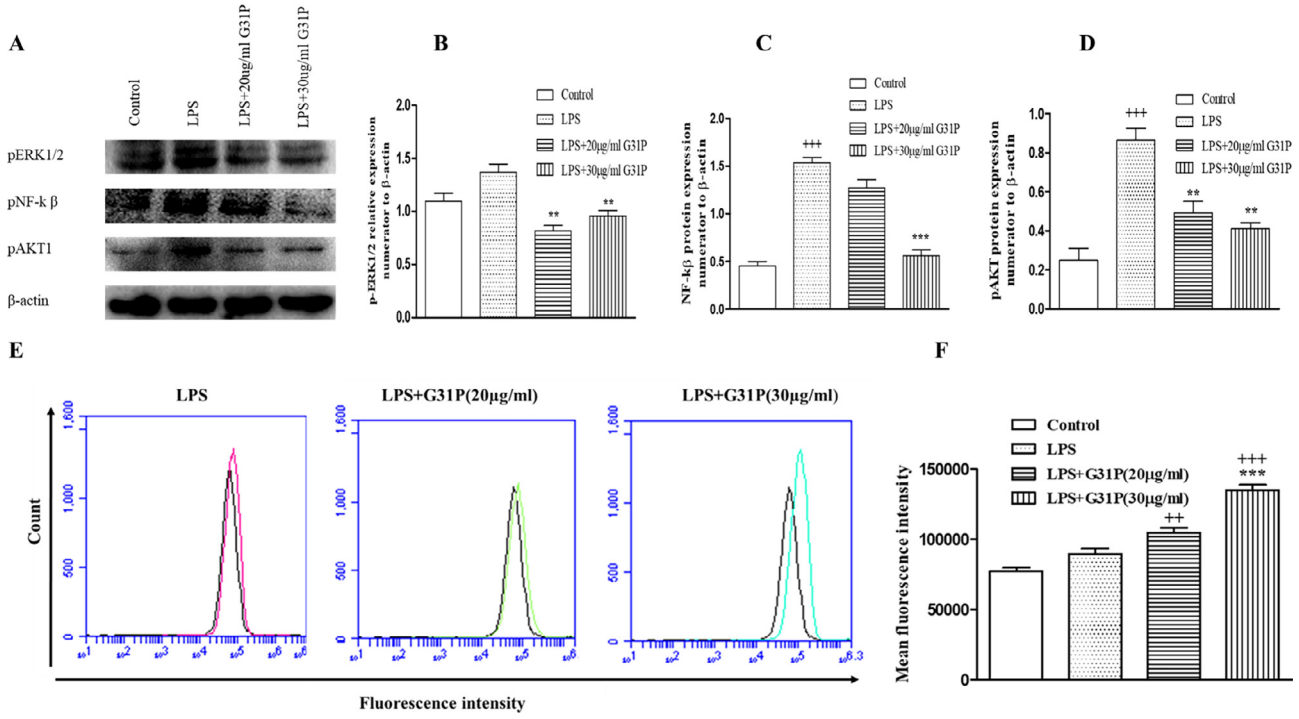

Fig. 5. G31P regulation of AKT1, ERK1/2, and ROS pathways. A. Representative western blot images. B-D. Relative pERK1/2, NF-kβ, and pAKT1 respectively, β-actin as internal reference. E. Visualized flow cytometry analysis of ROS expression. F. Mean fluorescence intensity of ROS expressed by THP-1 cells. All shown as mean ± SEM of independent triplicate experiments. $^{+++}p < 0.001$ vs Control. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ vs LPS induced group.

immune cells to the inflamed region. Similarly, TNF-α serves as chemoattractant for immune cells, and induces apoptosis and necrosis. It also enhances its own secretion, and the stimulation of IL-1β, IL-6 and IL-8 by immune cells and other tissues. Our treatment via binding to CXCR1/2, and reducing IL-8 secretion causes inhibition of all these cytokines, thereby promising as a therapeutic agent. On the contrary, IL-10 expression seems to be uninfluenced by G31P treatment, suggesting IL-10 maintains its regulatory role, for example as T reg modulator. The relatively elevated levels of IL-10 observed in this study is consistent with previous findings that IL-10 protects against the effects of IL-1β and TNF-α by reducing their expressions [36].

Inflammatory associated enzymes play crucial roles in regulating inflammation. We therefore assessed the effect of G31P on the expression of COX-2, MMP-2, and MMP-9. These enzymes are elevated during inflammation, but it has been shown that G31P treatment can reverse their expression, and contributes to controlling inflammatory reactions. It is however unclear whether G31P acts directly or indirectly on these enzymes. Possibly, the elevated IL-10 level, which has been shown to down-regulate COX-2, MMP-2, and MMP-9 could be partly an explanation for this observation [37]. It must be noted that the increased expression of IL-10 is not always protective as in some disease conditions its expression contributes to poor prognosis. Since the finding in this study is largely based on transcriptional analysis, it will be appropriate future investigations target the translational phase of these inflammatory related factors.

Transcriptional factors are key in eliciting the effect of inflammation, and their regulation remains potential therapeutic target. The effect of G31P on c-Fos, c-Jun, SP-1, and NF-kB were therefore determined. The regulatory role played by these factors have been reported in several studies. It has been established that the expression of COX-2, IL-1β, IL-6, IL-8 and TNF-α are regulated by the transcription factor NF-kβ [38,39]. This has been confirmed by the application of NF-kβ inhibitor, which elicits a corresponding decreased expression of these cytokines. Also, IL-8, TNF-α, and some cancers have been reported to be regulated by AP-1 transcription factor. AP-1 is a dimeric protein complex consisting of both c-Fos and c-Jun proteins. The Jun proteins family is made up of c-Jun, Jun-B, and Jun-D, while that of the Fos contains c-Fos, Fra-1, Fra-2, and Fos-B [40]. In this study, we focused on AP-1 heterodimer c-Fos-c-Jun complex. The c-Fos subunit, but not c-Jun, was significantly decreased by G31P. This observation suggests that G31P compromises the transcriptional activity of AP-1, hence the decline in the associated pro-inflammatory cytokines studied herein. These findings are in concordance with previous studies [41,42].

The transcription factor SP-1 has been reported to be activated by ERK1/2 [41]. However, ERK1/2 is also activated by monocyte colony stimulating factor. Previous findings revealed that ERK1/2 inhibition restrains the nuclear translocation of SP-1 [43]. Interestingly, in commensurate with past reports, G31P down-regulates both pAKT1 and pERK1/2 [26,28]. It was therefore expected to see diminishing expression of SP-1, but the reverse was observed. Possibly, there exist other SP-1 pathways which are not interrupted by G31P, and will be worth investigating, particularly at the translation level. LPS stimulation of THP-1 monocytes activates the production of ROS. There was insignificant increase of ROS in the LPS induced group, compared with Control group. However, G31P treatment was seen to promote ROS production in concentration-wise. There have been varied reports on the influence of ROS in inflammation. While some studies indicate its increment proportionally increases cytokine expression, others have reported that relatively appreciable levels of ROS contributes to reversing elevation of inflammatory cytokines [44–47]. Even though G31P treatment significantly increased ROS production in this study, it remains unclear whether this up-regulation contributed to the declining cytokines expression observed.

In summary, this study concludes that the IL-8 antagonist, G31P, via CXCR1 and CXCR2, arrests the expression of pro-inflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α. The inflammatory related enzymes COX-2, MMP2, and MMP-9 are inhibited following G31P treatment. AP-1 transcription function is mitigated via c-Fos down-regulation, but not c-Jun. Also, NF-kβ protein expression experienced a downward trend, while SP-1 transcription was upward. Furthermore, G31P down-regulates AKT1 and ERK1/2, and up-regulates ROS in THP-1 cells. Collectively, the findings of this study backed by previous reports suggest that IL-8 analogue, G31P, exhibits potent anti-inflammatory activity against LPS induced THP-1 monocytes, and promises therapeutic effect.

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Authors' contributions

WW and FL designed the study. WW, JW, MA, AE performed experiments. WW, IBY, MN performed data analysis. WW, SK, and EDK drafted the manuscript. FL, JC and JRG prepared G31P. FL supervised the study. All authors reviewed and approved the manuscript.

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