Chalcone Against Interferon Gamma (IFN-γ) Induced Oxidative Stress Indicate by Nitric Oxide Production

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A B S T R A C T

Interferon (IFN)-γ confers crucial immune surveillance positively for immune modulation, one of which is as antimicrobial such as antiviral replication, microbial killing, and MHC induction. Gamma interferon (IFN-γ) also a critical cytokine in host defense against salmonella infections. IFN-γ activation renders the macrophages capable of killing intracellular mycobacteria by overcoming the phagosome maturation block, nutrient deprivation and exposure to microbicidal effectors including nitric oxide (NO). Chalcones have been linked with immunomodulation, antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, and anti-diabetic activities. In this study, we aim to prove this speculation whether Chalcone can reduce oxidative inflammatory responses by targeting Nitric Oxide production. Changes in cell viability and cytotoxicity in murine macrophage RAW264.7 cells treated with hydrogen peroxide and chalcones were monitored. By using the sub-lethal dosages of these treatments, IFN-γ-induced the production of Nitric Oxide used as biomarker of oxidative stress. These results indicate that chalcone confers antioxidant activity against oxidative stress indicate by Nitric Oxide production.

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I. INTRODUCTION

Interferon (IFN)-γ confers crucial immune surveillance positively for immune modulation, one of which is as antimicrobial such as antiviral replication, microbial killing, and MHC induction. Focusing in microbial killing activity, IFN-γ can be both induced by bacteria and bacterial product. Endogenous IFN-γ production has been shown to play a protective role in the natural host response to several bacterial infections; and administration of exogenous IFN-γ is effective in the prevention and treatment of bacterial infections in numerous animal model systems (1). Gamma interferon (IFN-γ) also a critical cytokine in host defense against salmonella infections, but its role in phagocytic killing of intracellular Salmonella spp. has been investigated mainly in animal rather than human cells. This research measured the effect of recombinant IFN-γ(rIFN-γ) priming on bacterial internalization, intracellular killing, oxidative burst, and cytokine release during phagocytosis of Salmonella enterica serovar Typhimurium on human monocyte-derived macrophages (MDM) (2). IFN-γ activation renders the macrophages capable of killing intracellular mycobacteria by overcoming the phagosome maturation block, nutrient deprivation and exposure to microbicidal effectors including nitric oxide (NO) (3).
Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids.

Chalcones and their derivatives demonstrate wide range of biological activities such as anti-diabetic, anti-neoplastic, anti-hypertensive, anti-retroviral, anti-inflammatory, anti-parasital, anti-histaminic, anti-malarial, anti-oxidant, anti-fungal, anti-obesity, anti-platelet, anti-tubercular, immunosuppressant, anti-arrhythmic, hypnotic, anti-gout, anxiolytic, anti-spasmodic, anti-nociceptive, hypolipidemic, anti-filarial, anti-angiogenic, anti-protozoal, anti-bacterial, anti-steroidal, etc (4,5,6).

In this research we focus in antioxidant activity of Chalcone by neutralizing oxidative inflammatory responses by targeting Nitric Oxide production induced by IFN-γ.

II. RESULTS

![Chalcone Chemical Formula](image1)

Figure 1. Chalcone Chemical Formula

![Nitric Oxide Production](image2)

Figure 2. Interferon Gamma induced Nitric Oxide Production
Figure 3. Cell Viability of MTC treatment

Figure 4. Cell Cytotoxicity by MTC
III. DISCUSSION

Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids.

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All cell function abnormalities that will ultimately cause damage to tissue and organ function begins with the occurrence of oxidative stress, while oxidative stress can be neutralized by antioxidants. The discovery of types of antioxidant is still very necessary as everyone will have different reactions to any types of antioxidants. Focusing in antioxidant activity of Chalcone by neutralizing oxidative inflammatory responses by targeting Nitric Oxide production induced by IFN-γ, first we checked NO induction by IFN-γ. Then we also found that Chalcone don’t has high cytotoxicity as well as could maintain cells viability. Finally by sub lethal doze we found, Chalcone has proven could prevent oxidative inflammatory responses by targeting Nitric Oxide production induced by IFN-γ.

More research is needed to demonstrate the antioxidant effects of Chalcone. For example by looking at the effects of Chalcone in the NF-κB signaling pathway. Through transcription factor NF-κB which is critical for a wide range of processes such as immunity, inflammation, cell development, growth, and survival (7). It is activated by a variety of stimuli including cytokines, ionizing radiation, and oxidative stress(such as exposure to hydrogen peroxide) (8). Oxidative activation of NF-κB may involve the sequential activation of ASK1, SEK1, and JNK. Activated JNK induces the accumulation of β-TrCP protein, which facilitates the ubiquitination process of IκB protein (9).
IV. METHODS

Cell Line

Murine macrophage RAW264.7 cells (ATCC® CCL-10™) were grown and maintained on 10 cm plate in DMEM Medium 1640 (DMEM; Invitrogen Life Technologies, Rockville, MD), with L-glutamine and supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen Life Technologies), 50 units of penicillin, and 50 mg/ml of streptomycin.

Greece Reagen

The production of nitric oxide (NO) will be assessed as accumulation of nitrite (NO2-) in the medium using a colorimetric reaction with Griess Reagent. For Standard preparation, provide 7 step standards in 1 ml Eppendorf. Put 50 µl ddH2O in each Eppendorf, dilute 50 µl NaNO3 to get serial concentration. Make the 7th eppendorf as blank. Briefly after treatment, the culture supernatant will be collected. Put 50 µl supernatant and mix with equal number of reagent (1:1), either for standard and sample. After 5 ~ 10 minute when the colour change, the absorbance will measure at 540 nm using 96-well microplate reader.

MTT Assay

30000 cells/well will culture in 96-well plate at 37°C, 5% CO2. After 24h, cultures were treated with different experimental conditions: control, cytokines, ghrelin100mM, and a mixture of cytokines and CSE during 12, 48 and 72 hours. Culture supernatants were eliminated and 25 µl of MTT solution diluted at 5 mg/mL (Merck Chemicals Limited, Darmstandt, Germany), will add to each well. Samples will incubate for 90 minutes with 5% CO2 at 37°C and then wash with warm PBS. PBS was discarded and 100 µl of DMSO/well was added. Plate was covered and gently shaken during 20 minutes at RT. Absorbance was measure at 570 nm.

LDH Assay

Cell growth will be measured here. By using colorimetric assay (Cell Counting Kit-8; Dojindo Molecular Technologies, Kumamoto, Japan), we will determine cell viability according to the manufacturer’s instructions. By using a microplate reader (SpectraMax 340PC; Molecular Devices, Sunnyvale, CA) and in the absorbance at 450 nm, data will be analyzed by using Softmax Pro software (Molecular Devices). The relative growth rate will be normalized to the control group.

Statistical analysis. Values will express in mean ± standard deviation (SD). Groups will be compared by using Student’s two-tailed unpaired t test or one way ANOVA analysis followed by Dunnett post hoc test, as appropriate. These analyses will be performed by using GraphPad Prism 4 software (GraphPad Software, La Jolla, CA). Statistical significance is set at p<0.05.
V. REFERENCES


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Author contributions

D.I.K., CFL and CLC., conducted the experiments; D.I.K., CFL and CLC., designed the experiments; CFL. provided materials; D.I.K., D.A.S.,D.R.,M.A.,P.S.,F.R.,Sc.,E.S.,E.Q.R.,and H.I.wrote the paper; and all authors read and approved the manuscript.

Additional information

Competing financial interests: The authors disclose that they have no potential conflicts of interest.