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Original Research Article

Celastrol attenuates fMLP-induced superoxide anion generation, myeloperoxidase production, and elastase release by human neutrophils

Nipapan Malisorn*, Ammara Chaikan
Division of Pharmacology, Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani, Thailand

For correspondence:* ***Email:* *dr.nipapan@gmail.com;* ***Tel:*** *(+66)2-926-9716*

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Abstract

Purpose: *To investigate the anti-inflammatory effect of celastrol via attenuation of formyl-methionyl-leucyl-phenylalanine (fMLP)-induced superoxide generation, myeloperoxidase production, and elastase release by peripheral blood neutrophils.*
Methods: *Cytotoxicity of celastrol on human peripheral blood neutrophils was investigated using a 2H-tetrazolium hydroxide (XTT) assay. Human neutrophils were stimulated with 100-nM fMLP; the effect of celastrol on superoxide generation was determined via ferricytochrome C reduction, the effect on myeloperoxidase production by tetramethylbenzidine oxidation, and the effect on elastase activity by Boc-Ala-ONp hydrolysis.*
Results: *Treatment of human neutrophils with celastrol showed dose-dependent inhibition of fMLP-induced superoxide generation, myeloperoxidase production, and elastase release with half-maximal inhibitory concentration (IC_{50}) values of 5.9 ± 0.1, 1.9 ± 0.2, and 1.5 ± 0.1 µM, respectively.*
Conclusion: *These results indicate that celastrol possesses anti-inflammatory properties via attenuation of fMLP-induced superoxide generation, myeloperoxidase production, and elastase release by peripheral blood neutrophils.*

Keywords: *Celastrol, Anti-inflammatory, Superoxide anion generation, Myeloperoxidase, Neutrophil elastase*

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INTRODUCTION

Inflammation is a vital part of the immune response to insults ranging from trauma to infection. However, prolonged or unregulated inflammation contributes to the pathology of many diseases (e.g., arthritis [1], cancer [2], emphysema [3], and vasculitis [4]).

Neutrophils are crucial elements of cell-mediated immune responses, playing an important role in inflammation [5]. They are recruited to the site of damage within seconds and then destroy invading microorganisms by phagocytosis and digestion, which generate the toxic oxygen metabolite superoxide ($O_2^{\bullet-}$) from NADPH oxidase [6]. Thus, uncontrolled neutrophil activation produces excessive toxic oxygen metabolites that damage the host's own tissues

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[7]. Furthermore, neutrophil activation also liberates myeloperoxidase and elastase; myeloperoxidase catalyzes a halogenation reaction, generating a toxic end-product, hypochlorous acid, and elastase denatures elastin and collagen, which inhibits healing and the resolution of inflammation [8,9]. Obviously data showed that non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, could inhibit superoxide generation, myeloperoxidase production, and elastase release. Several studies have established that celastrol has therapeutic effect on inflammatory diseases including asthma, rheumatoid arthritis, and amyotrophic lateral sclerosis [10-12]. However, limited data exist regarding the effects of this compound on human neutrophil function.

The aim of this study was to determine whether celastrol can inhibit formyl-methionyl-leucyl-phenylalanine (fMLP)-induced inflammatory processes by peripheral blood neutrophils.

EXPERIMENTAL

Materials

All chemicals were obtained from Sigma Aldrich (USA) unless stated otherwise. Celastrol was purchased from TOCRIS Bioscience (USA).

Isolation of peripheral blood human neutrophils

This study was approved by ethical review board of Faculty of Medicine, Thammasat University, Thailand [13] with a certificate no. 074/2560. Human neutrophils were isolated by a discontinuous Percoll gradient method [14]. Neutrophils were re-suspended in RPMI 1640 for measurement of neutrophil cytotoxic assay or in phosphate buffered saline for measurement of superoxide generation, myeloperoxidase production, and elastase release.

Cytotoxicity assay

Neutrophils were treated with celastrol or indomethacin (final concentration of 0.1–1,000 μM) or RPMI 1640 (control) for 4 h at 37 °C. Then, 2H-tetrazolium hydroxide was added, and the mixture was incubated for 3 h at 37 °C. Cell viability was evaluated at 450 nm using a BioTex microplate reader [15]. Each reaction mixture was performed in triplicate. Percentage of cell cytotoxicity was convert from % cell viability, 100 – ((OD sample – OD medium) / (OD control – OD medium) x 100 %).

Determination of superoxide generation

Neutrophils were treated with celastrol or indomethacin (final concentration of 0.1–100 μM) or phosphate buffered saline (control) at 37 °C for 10 min. fMLP (100 nM) was then added, incubated at 37 °C for 10 min. Superoxide generation was determined via ferricytochrome C reduction at 550 nm using a BioTex microplate reader [14]. Results were reported as percent inhibition of superoxide.

Determination of myeloperoxidase production

Neutrophils were treated with celastrol or indomethacin (final concentration of 0.1–100 μM) or phosphate buffered saline (control) at 37 °C for 10 min. fMLP (100 nM) was then mixed and incubated at 37 °C for 10 min. Supernatants were treated with tetramethylbenzidine (TMB) mixture at 37 °C for 7 min. Myeloperoxidase production was measured via oxidation of tetramethylbenzidine and assessed at 450 nm using a BioTex microplate reader [16]. Results were reported as percent inhibition of myeloperoxidase production.

Evaluation of elastase release

Neutrophils were treated with celastrol or indomethacin (final concentration of 0.1–100 μM) or phosphate buffered saline (control) at 37 °C for 10 min. fMLP (100 nM) was then added, incubated at 37 °C for 10 min. The supernatants were treated with Boc-Ala-ONp at 37 °C for 20 min. Elastase activity was assayed via the hydrolysis of Boc-Ala-ONp at 405 nm using BioTex microplate reader [17]. Results were reported as percent inhibition of elastase release.

Statistical analysis

Results are reported as mean ± SEM. Statistical significance ($p < 0.05$) was detected by one-way ANOVA using GraphPad Prism 8.1.2 (GraphPad Prism, CA), followed by Student-Newman-Keuls analysis.

RESULTS

Cytotoxicity

The incubation of human neutrophils with 0.1–100 μM of celastrol or indomethacin caused no cytotoxic effects. However, at 1,000 μM, both celastrol and indomethacin caused cytotoxicity (26.8 ± 2.0 % and 3.8 ± 0.4 %, respectively) (Figure 1).

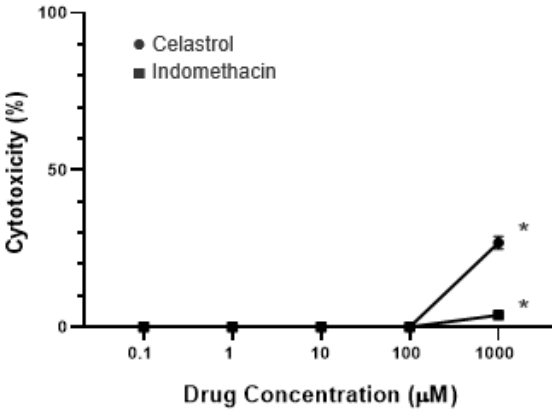

Figure 1: Cytotoxicity of celastrol or indomethacin (0.1–1,000 μM) on human peripheral blood neutrophils. Results are reported as mean ± SEM (n = 7); *p < 0.05 when compared with control cells

Superoxide generation

Celastrol suppressed fMLP-induced superoxide generation in a dose-dependent manner and did so more strongly (IC_{50} = 5.9 ± 1.0 μM) than indomethacin (IC_{50}= 16.4 ± 3.5 μM) (Figure 2); this effect was significant at concentrations of 1–10 μM (p < 0.05). However, at 100 μM, indomethacin exhibited significantly stronger inhibition of fMLP - induced superoxide generation than celastrol (p < 0.05; Figure 2).

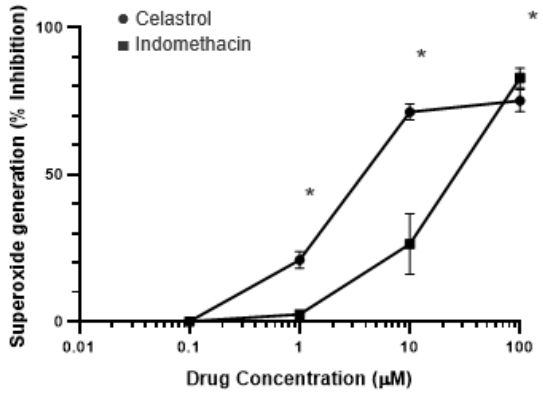

Figure 2: Inhibitory effect of celastrol and indomethacin on fMLP-induced superoxide generation in human peripheral blood neutrophils. Results are reported as mean ± SEM (n = 7); *p < 0.05 for comparison of indomethacin vs. celastrol

Myeloperoxidase production

Celastrol inhibited fMLP-induced myeloperoxidase production in a dose-dependent manner that was stronger than indomethacin (IC_{50}: 1.9 ± 0.2 μM, 50.7 ± 1.3 μM, respectively) (Figure 3), and these differences were statistically significant at concentrations of 1–100 μM (p < 0.05).

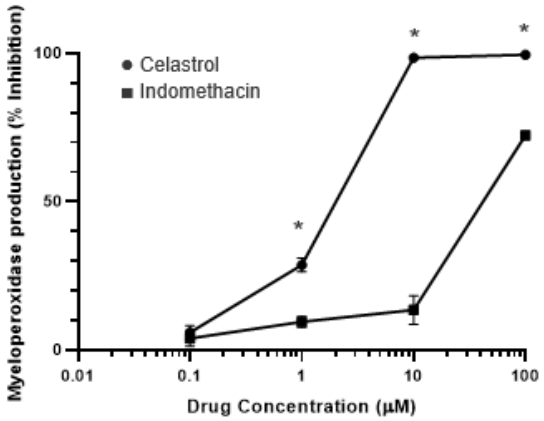

Figure 3: Inhibitory effect of celastrol on fMLP-induced myeloperoxidase production in human peripheral blood neutrophils. Results are reported as mean ± SEM (n = 7); *p < 0.05 for comparison of indomethacin vs. celastrol

Elastase release

Celastrol suppressed fMLP-induced elastase release in a dose-dependent manner. Celastrol had a stronger inhibition (IC_{50} = 1.5 ± 0.1 μM) than indomethacin (IC_{50} = 15.7 ± 2.7 μM) (Figure 4), and the inhibitory effect of celastrol was significantly better than indomethacin at concentrations of 0.1–100 μM (p < 0.05).

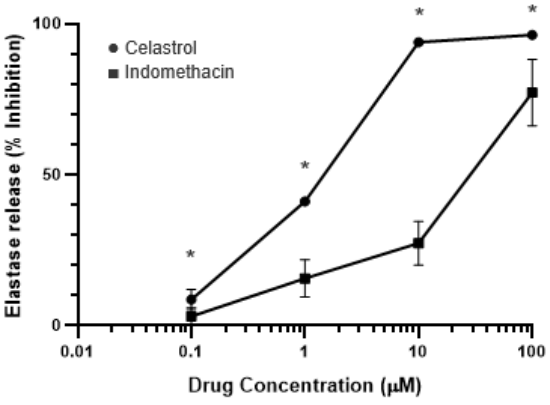

Figure 4: Inhibitory effect of celastrol on fMLP-induced elastase release in human peripheral blood neutrophils. Results are reported as mean ± SEM (n = 7); *p < 0.05 for comparison of indomethacin vs. celastrol

DISCUSSION

In inflammation, neutrophils are recruited to the damaged site by fMLP; upon arrival, neutrophils degranulate, generating superoxide, myeloperoxidase, and elastase to destroy pathogens. fMLP is a potent chemotactic substance [19] that attracts and activates neutrophils, which, in turn, liberate superoxide

through NADPH oxidase activity [20]. Unregulated, this degranulation contributes to tissue destruction [18]. Although previous work demonstrates that celastrol completely inhibits NADPH oxidase enzymes [21], our study showed that celastrol inhibits fMLP-induced superoxide generation by peripheral blood neutrophils is dose-dependent, with maximum inhibitory effects achieved at a concentration of 100 μM.

Myeloperoxidase, regarded as a crucial biomarker of inflammatory diseases (e.g., as rheumatoid arthritis, sinusitis and colitis [24-26]), produces many reactive oxidants, such as hypochlorous acid, by consuming hydrogen peroxide and chloride anion [23]. Although nonsteroidal anti-inflammatory drugs (NSAIDs), including indomethacin, diclofenac, naproxen, and piroxicam, inhibit myeloperoxidase [27], our study showed that celastrol also inhibits fMLP - induced myeloperoxidase production in a dose-dependent manner.

Elastase [28], another causative factor in inflammatory disease, is also suppressed by NSAIDs. Here, we demonstrated that celastrol too suppresses neutrophil elastase release in a concentration-dependent manner.

CONCLUSION

In human neutrophils, celastrol significantly inhibited fMLP-induced superoxide generation, myeloperoxidase production, and elastase release in a dose-dependent manner. These effects might be attributed, at least in part, to its anti-inflammatory activity. Therefore, celastrol may be a promising candidate substance for further development as an anti-inflammatory therapy.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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