

Exploring the Effects of Theophylline on Neutrophil Function in Inflammatory Diseases

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Abstract:

Neutrophils play a key role in the human immune system as the first cells to migrate to sites of inflammation. Several common respiratory diseases, such as chronic obstructive pulmonary disease (COPD) and asthma, are characterized by excessive chemotaxis and damaging apoptosis of neutrophils around infection. Theophylline, one potential therapeutic candidate, was employed to examine drug effects on neutrophil function. The chemotactic behaviors of neutrophils were monitored using a microfluidic platform after incubation with various concentrations of theophylline for different time periods. Theophylline worked to limit the motility of neutrophils in small concentrations and long incubation times; however, there was no influence on the polarization of neutrophils. Meanwhile, theophylline induced a tremendous decrease in neutrophil viability based on colorimetric assay. This work provides new insights on how theophylline affects neutrophil function *in vitro*, potentially guiding drug application for the treatment of neutrophilic inflammation *in vivo*.

Introduction:

Chemotaxis is a dynamic cellular process by which cells migrate to inflammatory areas in the presence of chemical gradients composed of signaling molecules called chemokines [1]. Abnormal excessive chemotaxis and failure in apoptotic pathways are the main contributors for neutrophil accumulation around infection sites. Neutrophilic inflammation is resistant or poorly responsive to some traditional drugs targeted for chronic respiratory diseases, such as corticosteroids. Theophylline has been successfully utilized as a pharmaceutical treatment for

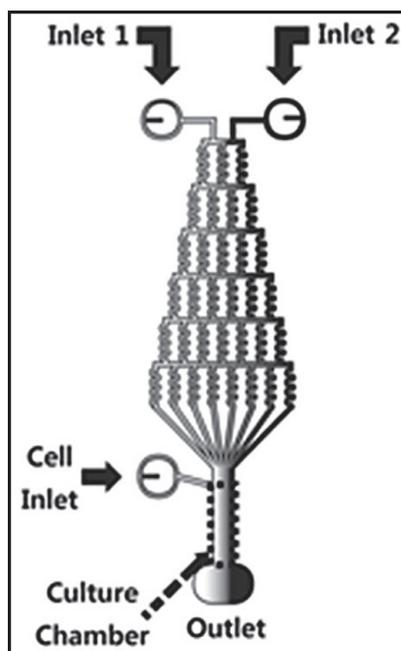


Figure 1: Schematic of the device in this work.

neutrophilic inflammation, but the detailed mechanisms are not clear. In this work, the drug effects of theophylline on neutrophil function were studied to explain the mechanism of drug-cell interaction and provide direction for the design of novel anti-inflammatory approaches.

Microfluidic technology is unique in its ability to mimic *in vivo* process *in vitro* by maintaining a flow vital to observing biological processes. More importantly, microfluidic devices are able to provide stable chemokine gradients with high spatiotemporal resolution and quantitative data for describing chemotactic behaviors in different conditions. A gradient microfluidic device containing parallel serpentine channels and a cell culture chamber was employed in this study (Figure 1) [2].

Method:

Microfluidic device molds were fabricated through photolithography methods with SU-8 photoresist spun onto a silicon wafer. Polydimethylsiloxane (PDMS) was poured on the mold and cured overnight, followed by permanently bonding PDMS and glass slides using oxygen plasma.

Human blood samples were layered on separation medium and centrifuged to isolate neutrophils. Cell mixtures were washed with Hank's balanced salt solution (HBSS) buffer and cell lysis buffer, then pure neutrophils were counted and diluted to the appropriate concentration.

Prior to injecting neutrophils into the device, the cell culture chamber was incubated with fibronectin. Next 5-10 μL of neutrophil suspension ($3-5 \times 10^6$ cells/mL) in HBSS buffer was introduced into the device (Figure 2). Three different

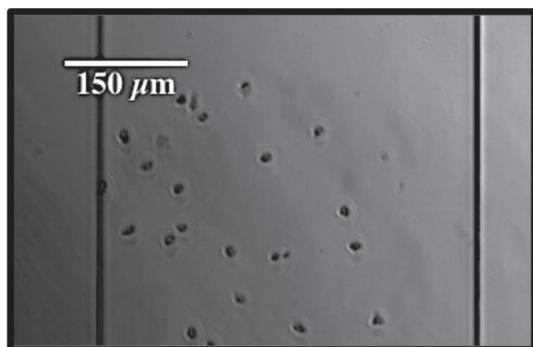


Figure 2: Neutrophils incubated on the bottom of the cell culture chamber.

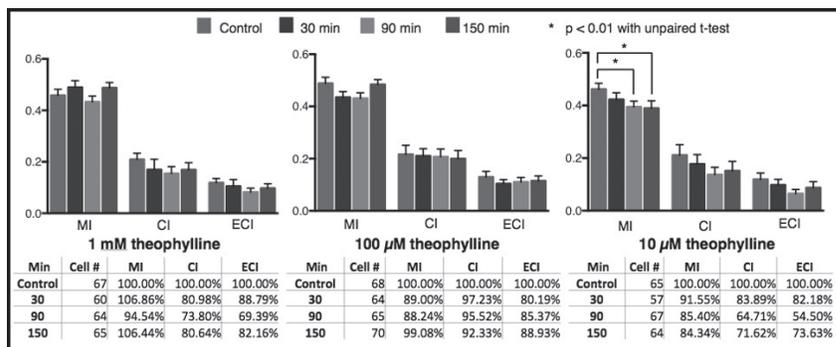


Figure 3: Results summary of neutrophil chemotaxis after incubation with 1 mM, 100 μM and 10 μM theophylline for different lengths of times. Error bars indicate standard deviation.

theophylline concentrations were tested: 1 mM, 100 μM, and 10 μM, for durations of 30 minutes, 90 minutes, or 150 minutes.. A chemokine gradient was created by mixing buffer and 10 mg/mL interleukin-8 (IL-8) solution in the serpentine channels under 100 μL/h flow rate.

Neutrophil activity was assessed for 20 minutes by calculating the motility index (MI), chemotactic index (CI), and effective chemotactic index (ECI) with Metamorph software. MI quantifies the overall movement of the neutrophils, and CI analyzes how much cells moved in the gradient direction. ECI is the product of MI and CI, examining the effectiveness of neutrophil migration.

The cytotoxicity of theophylline was monitored using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In the 96-well plate, 100 μL of neutrophil suspension (6 × 10⁵ cells/mL) was incubated with each drug concentration for the various time periods. After incubation, neutrophils were mixed with 100 μL MTT solution (0.5 mg/mL) for two hours to produce precipitation, and 100 μL DMSO was added to dissolve otherwise insoluble crystals. Finally, optical density was measured at 570 nm, with 655 nm as reference, using a microplate reader.

Results and Conclusions:

As shown in Figure 3, 10 μM theophylline and long incubation times (90 and 150 minutes) induced a significant decrease in motility of neutrophils; however, larger concentrations (1 mM and 100 μM) had no impact on MI. There was no influence on the polarization and effectiveness of neutrophil chemotaxis under any of the conditions. Meanwhile, theophylline caused an obvious drop in neutrophil viability based on the MTT assay in a time-dependent manner. Cell viability was reduced to 50% compared to the initial viability after 150 minutes of incubation for all concentrations of theophylline.

These preliminary results displayed that theophylline is not an effective regulator of neutrophil chemotaxis except at the lowest

concentration considered, contradicting previous findings that theophylline attenuated neutrophil chemotaxis in the traditional chamber-based assays [3]. A reasonable explanation is that theophylline has slight effects on neutrophil chemotaxis in short term. Instead, high cytotoxicity was observed, implying that theophylline acts as a potent treatment by inducing cell death, which was not taken into account in current literature (Figure 4). This work supplied a better understanding of the relationship between drug effects and neutrophil function relevant in multiple diseases.

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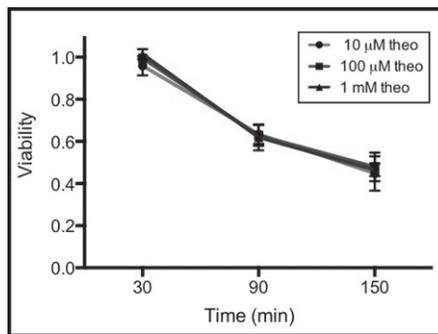


Figure 4: Results of cell viability using MTT assay.