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Karolinska Institutet CBCS CHEMICAL BIOLOGY CONSORTIUM SWEDEN UNIVERSITY OF GOTHENBURG

Identification of small molecule modulators of reactive oxygen release in TNF-α primed primary human neutrophils.

Introduction

Neutrophils are components of the innate immune system with an important role in the protection against external microorganisms. Neutrophils in circulating blood are normally in a resting state, but a number of inflammatory mediators can change them into an active state by a priming procedure. Activation triggers the adhesion and penetration of neutrophils into inflamed tissue, where reactive oxygen species (ROS) are released through the activation of NADPH oxidase.

Tumor necrosis factor alpha (TNF-α) is one of the earliest cytokines produced at an inflammatory site. TNF-α itself does not activate NADPH oxidase in neutrophils to a great extent, but acts as a priming agent. Upon addition of the actin filament disrupting agent cytochalasin B to TNF-α primed neutrophils, a burst of released ROS is observed, with a timing and magnitude profile resembling that observed following stimulation of pro-inflammatory GPCRs expressed on the neutrophil surface.

We took advantage of this phenomenon and developed a screening assay aiming at identifying modulators of ROS release in TNF-α primed primary human neutrophils, with the goal to find novel GPCR modulators amongst the hits.

Experimental procedure

Freshly isolated primary human neutrophils are first incubated with TNF-α. The cells are then triggered with cytochalasin B. The produced superoxide anions are measured by a chemiluminescence technique based on isoluminol using the single photon counting camera of the Hamamatsu FDSS 7000 EX.

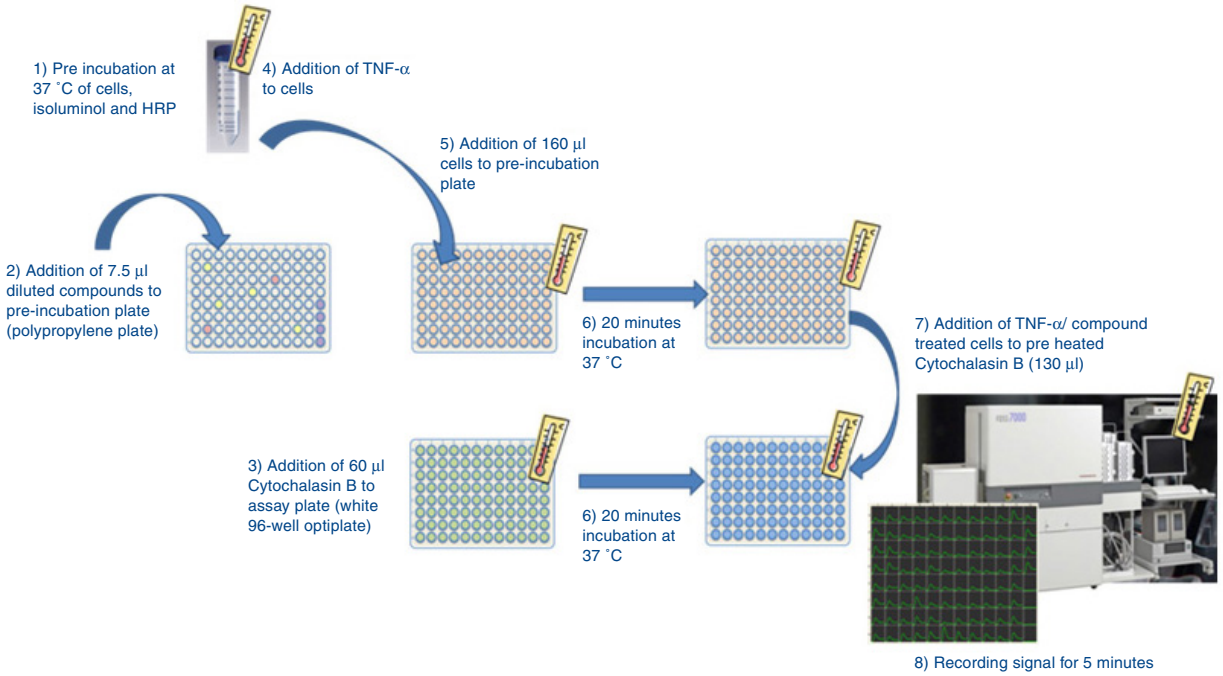
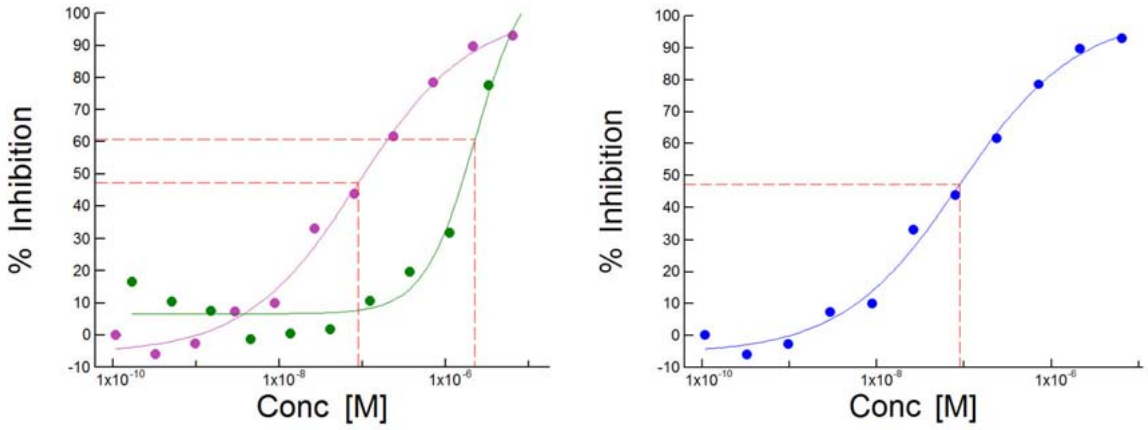


Fig 1 : Screening Assay Logistics

Results

A total of 7000 compounds were tested at a compound concentration of 1.3 μM. MAPK (p38) and PI3K inhibitors are known to modulate several of the TNF-α induced effects. SB203580, a p38 inhibitor, and LY294002, a PI3K inhibitor, were used as reference compounds (Cf Fig 2).



SB203580 IC_{50} 9 nM
LY294002 IC_{50} up to 1.6 μM

One of the most potent hits
SB202190 IC_{50} 7 nM

Fig 2 : IC_{50} curve of reference compound

Conclusions

We demonstrated the successful transfer of a screening method to identify novel molecular targets (GPCR modulators, etc.) in inflammatory responses from a tube-based to a microplate-based format, allowing to get closer to a HTS method. A 7000 compounds small molecule library was screened in order to identify modulators of ROS release in TNF-α primed primary human neutrophils: 74 compounds were identified as inhibitors of ROS release in the experimental system.

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