Use of novel inhibitor molecules as potential clinical agents for inflammation

Bhavana Gupta - Stage 3 Biomedical Science, b.gupta@ncl.ac.uk | Supervisor: Professor Michael Taggart

Aims

We aimed to test the anti-inflammatory properties of four IKB kinase (IKK) inhibitors provided by GlaxoSmithKline on WISH cells, HeLa cell derivatives that are used as human amnion cell models.

Introduction

Pre-term labor presents a substantial health burden to newborn babies and may predispose them amongst other things to infections, respiratory distress of the newborn and neurological problems. The great concern is that the cause remains largely unknown.

It is known that an inflammatory environment develops during human pregnancy in uteroplacental tissue. This has a stimulatory effect on the production of cytokines such as TNF-α and IL-1β which activate the canonical pathway of Nfκβ. This results in a signal cascade that leads to the alteration of the gene transcription levels of certain proteins.

One such protein is cyclo-oxygenase 2 (COX-2), also known as prostaglandin H synthase 2. The levels of COX-2 increase and this protein in turn upregulates the production of prostaglandins which are pro-contractile. This results in the induction of pre-term labor.

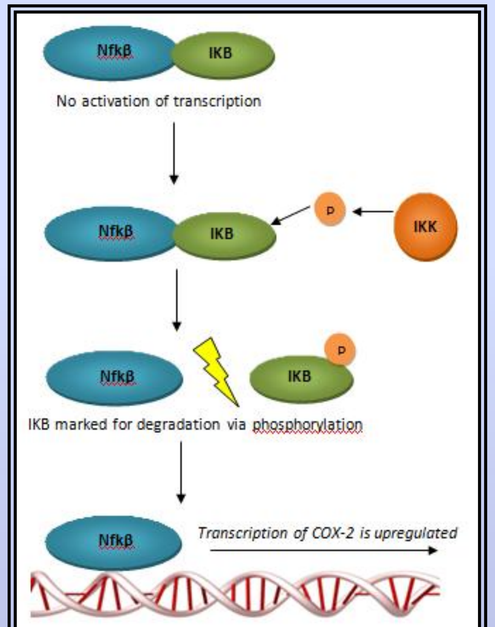
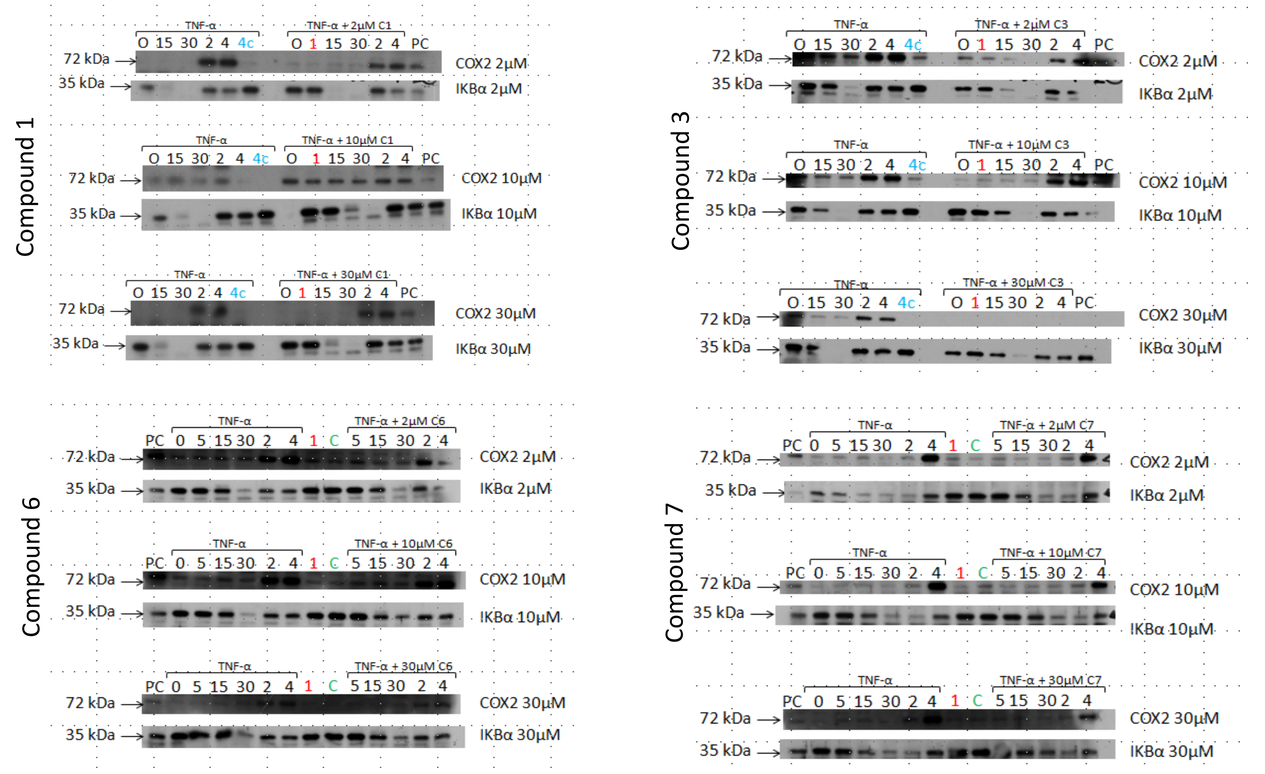


Figure 1. Canonical pathway of Nfκβ.

Methods

We performed time-course cell treatment experiments on WISH cells in 12-well culture plates – the drugs were tested at three different concentrations (2μM, 10μM, and 30μM). Protein was then collected from the lysed cells and protein assays were carried out to calculate the amount of protein to be loaded for the Western blots. Western blots were run and the resulting membrane was immunoblotted to tag the COX-2 and IkBα.

Results



The results show that addition of Compounds 1 and 3 to the cells stimulated with TNF-α does not have an inhibitory effect as the intensity of the protein bands before and after the addition of the inhibitors appear to be equal at the different time points.
Compounds 6 and 7 successfully downregulated the production of COX-2 at the time points 2 and 4 hours as the protein band became lighter after the addition of the compounds, signaling a decreased amount of protein. Also the levels of IkBα increased at these time points as the degradation of this protein was downregulated.

Conclusion

It was determined that Compounds 1 and 3 do not have a significant inhibitory effect although Compounds 6 and 7 were able to effectively inhibit the production of COX-2 and thus could possibly reduce inflammation in vivo. Hence, the latter compounds may have potential for clinical use.

We propose to repeat the experiment with Compounds 6 and 7 using primary myometrial cells (uterine smooth muscle cells) as unlike WISH cells, this cell type is uterine in nature.

References
Lappas, M., Rice, GE. (2006) 'The role and regulation of the nuclear factor kappa B signaling pathway in human labour,' *Placenta*, 28(5-6), pp.543-56.

Special thanks to Professor Michael Taggart, Julie Taggart, and Rachael Watson for their supervision and support throughout the project, as well as to the Reproductive and Vascular Biology Group and Newcastle University for funding my project.