A165 EFFECT OF THE PEG COMPONENT OF CERTOLIZUMAB PEGOL ON CALCIUM FLUX IN CELLULAR SYSTEMS

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Background and objectives Certolizumab pegol (the only polyethylene glycolylated (PEG) Fab' anti-tumour necrosis factor (CZP)) has a very low incidence of injection site pain (ISP) in clinical studies. This could be due to inhibition of mast cell degranulation by the CZP PEG component. Mast cells could be involved in mediating ISP: they are present at high numbers in the skin and can rapidly secrete inflammatory mediators in preformed granules. Changes in Ca^{2+} flux are primary indicators of cell activation, and mast cell degranulation is preceded by Ca^{2+} flux into the cell. PEG binds to metal ions (in particular Ca^{2+}). The aim of this study was therefore to determine if PEG could inhibit Ca^{2+} flux in a cellular system.

Materials and methods Peripheral blood monocytes, isolated using MACS beads, were incubated with the fluorophore Fluo-4. Ca^{2+} flux was measured using flow cytometry by detecting the Fluo-4 emission at 515–535 m. Ionomycin was added at 2 $\mu\text{g}/\text{ml}$ and the emission measured over a 4 min period relative to background (assessed prior to ionomycin addition). The effect of a range of concentrations of the 40 kDa PEG component of CZP on Ca^{2+} flux induced by ionomycin was assessed. The effect of PEG on an ionomycin-induced Ca^{2+} flux in cultured mast cells was determined by a similar method but flux was measured using a fluorimeter.

Results Ionomycin caused a dramatic flux of Ca^{2+} in the monocytes. PEG caused a dose-dependent inhibition of Ca^{2+} flux over a range of concentrations from a minimum of 40 mg/ml. This is a physiological concentration of PEG as the equivalent local concentration of PEG which is injected into patients is 88.9 mg/ml. The inhibitory concentration (IC_{50}) for inhibition of the Ca^{2+} flux caused by PEG was around 10 mg/ml. The maximum inhibition observed was 84.2% obtained at 40 mg/ml, with the effect titrating out around 1 mg/ml. In mast cells the IC_{50} was around 11 mg/ml.

Conclusion The PEG component of CZP inhibits Ca^{2+} flux in monocytes and mast cells at a concentration relevant at the site of injection. This inhibition of Ca^{2+} flux could potentially explain the low levels of ISP observed with CZP in the clinic. This effect would only be observed at the site of injection as systemic concentrations of the drug are below the levels where an effect is seen.