

P007 Inhibition of ADAMTS-4/-5 by N-terminal N-TIMP-3 mutants

Ngee Han Lim¹, Masahide Kashiwagi¹, Shuo Wei², Keith Brew², Hideaki Nagase¹

¹Imperial College, UK ²Florida Atlantic University, USA

Osteoarthritis (OA) is a multifactorial disease which results in the destruction of cartilage. Aggrecan, the major cartilage proteoglycan, can be cleaved at several sites by both the matrix metalloproteinases (MMPs) and the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family of enzymes. Their relative roles in the progression of OA are unclear. We have previously shown that ADAMTS-4 and ADAMTS-5 are inhibited by the N-terminal domain of tissue inhibitor of metalloproteinase (N-TIMP)-3 but not by TIMP-1 or TIMP-2. Furthermore, only N-TIMP-3 was able to inhibit aggrecan degradation in an interleukin -1 α (IL-1 α)-stimulated model of cartilage destruction. Here we report the inhibition of ADAMTS-4 and ADAMTS-5 by three mutants of N-TIMP-3 with modifications at the N-terminus, Thr² \rightarrow Gly (T2G), (-1A) and (-2A) (with one and two additional alanines at the N-terminus respectively), which do not inhibit MMPs (apparent inhibition constants [$K_{i(\text{app})}$] for MMPs $>1 \mu\text{M}$). These mutants have a $K_{i(\text{app})}$ of less than 60 nM, against ADAMTS-4 and ADAMTS-5, with (-2A) having a 35-fold preference for ADAMTS-5 over ADAMTS-4. We also show that these mutants are still effective in inhibiting aggrecan degradation in IL-1 α -stimulated cartilage. These mutants provide a unique tool to ascertain the contributions of the different proteases in the human disease process.