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Potential clinical implications of recent MMP inhibitor design strategies

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Abstract

Analysis of MMP expression profiles in various pathologies correlated their presence in promoting disease progression. Drugs were designed to inhibit MMPs in an extreme manner by chelating the active site zinc ion. This approach did not distinguish between the 24 members of the MMP family and had devastating consequences during clinical trials. Subsequent knockout mouse studies showed that some MMPs are beneficial in regulating tumor growth, metastasis and indirectly stimulating the immune system. The broad-spectrum inhibitor approach was rethought and modified in order to increase specificity by taking into account the non-conserved secondary binding sites or differences in structures within MMPs and also generating antibodies. These showed interesting results *in vitro* and *in vivo*. The recent technological advances that allow us to better understand the function and structure of MMPs are aiding in the development of selective inhibitors.

Keywords

Matrix metalloproteinase (MMP); selective inhibitor; exosite; collagen; antibody therapy; triple-helical peptide

Matrix metalloproteinases (MMPs) have the ability to degrade the components of the extracellular matrix and are therefore a key participant in the homeostasis of tissue remodeling [1]. Overexpression of this family of zinc-dependent endopeptidases has been correlated to various diseases including cancer, in particular tumor angiogenesis and metastasis, osteoarthritis, inflammation, and vascular disease [2–4]. The natural balance of MMP activities is ensured by generally low enzyme expression, enzyme production as a zymogen, and the presence of tissue inhibitor of metalloproteinases (TIMPs). When the

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regulatory system is compromised during diseases, it is essential to reestablish the equilibrium. The pharmaceutical industry focused its efforts to identify various therapeutic vectors targeting MMPs. *In vivo* data indicated that MMP inhibition could indeed be a powerful tool in fighting cancer. These compelling findings lead to clinical trials of broad-spectrum MMP inhibitors, utilizing hydroxamic acids which chelated the active site Zn^{2+} in the MMP catalytic domain [5]. Batimastat (British Biotech), marimastat (British Biotech), and prinomastat (Agouron) were initially investigated. However, their lack of selectivity resulted in musculoskeletal syndrome (**MSS**) and inflammation. It became evident that inhibiting MMPs nonspecifically was not desirable. More recently, the non-hydroxamate AZD1236 ((5S)-5-[[4-(5-chloropyridin-2-yl)oxypiperidin-1-yl]sulfonylmethyl]-5-methylimidazolidine-2,4-dione; AstraZeneca), an MMP-9 and MMP-12 inhibitor undergoing a phase II randomized controlled 6 weeks trial on moderate to severe chronic obstructive pulmonary disease (COPD), showed an acceptable safety profile. However, the therapeutic efficacy could not be demonstrated in such a short trial [6]. FP-025 (Forsee Pharmaceutical), another non-hydroxamate-based inhibitor, showed selectivity for MMP-12 and high potency for the treatment of asthma and COPD. A randomized, placebo controlled, single and multiple ascending dose study in healthy subjects is currently ongoing to assess the safety, tolerability, and pharmacokinetics.

Several companies developed new molecules with better selectivity towards MMP-13 by targeting the S_1' subsite situated in the catalytic domain. The pyrimidinedione derivatives (Pfizer) showed potency in treating osteoarthritis in rabbit and dog models [7]. ALS 1-0635 (Alantos Pharmaceuticals) also presented promising results in treating osteoarthritis without inducing MSS in animal studies [8]. However, no clinical studies have appeared for any of these compounds presently, possibly due to solubility issues. High-throughput screening performed in our laboratory identified a new class of inhibitors for MMP-13, compounds Q, Q1, and Q2 [9]. These compounds bind to not only the S_1' but also the S_1/S_2^* subsite. The most attractive features of these compounds are that they do not inhibit MMP-1 and ADAM17, which lead to MSS, and they do not inhibit MMP-8, which shows very high selectivity. Although their affinity for MMP-13 was showed to be 10–100 times lower than compounds from Pfizer and Aventis, we suggest that this could be resolved by increasing the size of the Q, Q1, and/or Q2 scaffolds. A similar approach was recently described using a thienol[2,3-d]pyrimidine scaffold and extension into the S_1'' subsite of MMP-13 [10]. Compounds Q, Q1, and Q2 were also highly selective when tested against a panel of 30 proteases, which, in combination with a good cytochrome P450 inhibition profile, suggested low off-target toxicity and drug-drug interactions in humans [11].

Screening using a human Fab display phage library allowed the identification of DX-2400, a selective, fully human MT1-MMP inhibitory antibody [12]. In mouse models, DX-2400 was found to inhibit MT1-MMP activity preventing MDA-MB-231 primary tumor growth as well as metastasis [12]. Hybridoma technology against the human MMP-9 catalytic domain allowed the generation of murine monoclonal antibody REGA-3G12 [13]. REGA-3G12 recognized the Trp116 to Lys214 region of MMP-9, located in CAT domain but not part of the Zn^{2+} binding site [13]. The most interesting feature of REGA-3G12 is its ability to discriminate between MMP-9 and MMP-2. Antibodies for MT1-MMP also showed great

selectivity *in vitro* and *in vivo* [14]. Other groups developed an anti-MMP-13 antibody, based on a 3D structure in addition to the amino acid sequence, which allowed inhibition of active MMP-13 without interfering with its latent form or other MMPs [15]. Mouse antibodies towards synthetic organic ligands bound to a metal ion (Zinc-Tripod), which mimics structural and chemical motifs of the relatively exposed catalytic Zn^{2+} -His machinery in the active MMP, were recently generated. A second immunization against the full length MMP induced *in vivo* affinity maturation towards the native conformation of the catalytic site and additional surface epitopes presented in the whole enzyme. The produced antibody, SDS4, showed selectivity for MMP-2 and 9 and demonstrated therapeutic potential in an inflammatory bowel disease animal model [16]. This mode of inhibition displayed promising pre-clinical results and will need to be subject to clinical studies. So far, no clinical trial has yet been initiated for any of the antibodies described above. To our knowledge, GS-5745 (Gilead Science), a monoclonal anti-MMP-9, is the only antibody investigated by three phase I and one phase II clinical trials worldwide. It is currently evaluated for its safety, tolerability, pharmacokinetics, pharmacodynamics, and its therapeutic potency alone or in combination with chemotherapy in patients with advanced solid tumors that are refractory to standard therapy. One of the main concerns in using antibodies is their stability as they are subject to proteolysis and may be removed from the circulation rapidly. Another issue is administration, as the parenteral route reduces convenience in patient treatment [17].

Another strategy focuses on designing inhibitors based on substrates such as collagen. In order to add sequence diversity and eliminate off target interactions with non-collagenolytic proteases, a zinc-binding group was incorporated within a collagen-model THP. The phosphinic triple-helical peptide (THP) $\alpha 1(\text{V})\text{Gly}\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}\text{Val}$ [mep14,32,Flp15,33] was found to inhibit MMP-2 and MMP-9 selectively. In a mouse model of post-myocardial infarction, citrate synthase processing by MMP-9 was inhibited with $\alpha 1(\text{V})\text{Gly}\Psi\{\text{PO}_2\text{H}-\text{CH}_2\}\text{Val}$ [mep14,32,Flp15,33] [18], resulting in improved mitochondrial function. THPs are less susceptible to general proteolysis than peptides and other folded proteins and thus offer favorable pharmacokinetics. The primary disadvantage of this class of inhibitors is the cost of production.

Of the numerous MMPIs that have been proposed in the past 20 years, only a few are still investigated. Despite encouraging pre-clinical data, the design and development of selective MMP inhibitors remains at early stages. The major failure of the first generation of broad-spectrum inhibitors greatly undermined the continued evaluation of MMPs as disease targets. Through the use of MMP knockout mice, the main focus is now to be able to design inhibitors targeting detrimental MMPs without interfering with those which have an important role in preventing the progression of diseases. However, the high homology within the MMP family impedes the advancement in specific inhibitor development. Proteomic screens have provided key information on individual MMP degradomes in various biological systems as well as specific cleavage sites of their substrates. The recent development of powerful proteomic techniques will (a) allow the determination of the role of each MMP as a modulator in biological processes, (b) distinguish between detrimental and essential MMPs, and (c) enable a more refined approach for inhibitor design, based on substrate binding specificity [19]. Although *in vitro* and *in vivo* studies have showed encouraging results,

inhibitor stability and bioavailability remain problematic. The later may imply higher dose administration, which in turn leads to toxicity. One may consider a delivery system that is targeted to specific tissues in order to bypass toxicity due to a high dosage. Better imaging tools to assess *in vivo* MMP expression and activity during different stages of a disease will also be essential in order to optimize the spacio-temporal use of inhibitors. Initial clinical trials were faulty in that inhibitors were tested in late stage cancers, whereas animal data was obtained during cancer initiation. Timing has to be taken under consideration, and entry criteria for clinical trials ought to be at earlier stages in cancer treatment in order to match animal data. A much greater understanding of MMP structure and function remain essential but already the new generation of selective inhibitors shows great promise.

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