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MMP Inhibitors: Past, present and future

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1. ABSTRACT

Development of inhibitors of (MMPs) has been fraught with challenges. Early compounds largely failed due to poor selectivity and bioavailability. Dose-limiting side effects, off-target interactions, and improperly designed clinical trials significantly impeded clinical success. As information becomes available and technology evolves, tools to combat these obstacles have been developed. Improved methods for high throughput screening and drug design have led to identification of compounds exhibiting high potency, binding affinity, and favorable pharmacokinetic profiles. Current research into MMP inhibitors employs innovative approaches for drug delivery methods and allosteric inhibitors. Such innovation is key for development of clinically successful compounds.

2. PEPTIDOMIMETICS

The first inhibitors of matrix metalloproteinases (MMPs) were investigated for use in cancer therapy. During the early nineties, it had been established that MMPs can influence the tumor environment by promoting angiogenesis, tumor growth, and metastasis (1, 2). This marked a turning point in the field of Cancer Res, as cancer drug discovery had traditionally focused on compounds which kill cancer cells (which remains the case today). But because most forms of aggressive cancers remain resistant to chemotherapeutics, metastasis remains responsible for 90% of patient deaths (3). Thus MMPs were viewed as a druggable target to contain primary tumors and delay disease progression. During this period of early optimism, nearly every company in the pharmaceutical industry invested in identification of compounds which could inhibit MMP activity.

MMPs are endopeptidases with a zinc ion in the catalytic domain required for coordination of catalysis. The majority of small molecule compounds clinically bind in the active site and inhibited the enzyme by chelating this catalytic zinc (4). The first MMP inhibitors tested clinically were soluble peptides designed to mimic the amino acids of the endogenous MMP ligand, collagen. Batimastat was the first of these peptidomimetic inhibitors to reach clinical trials for cancer. This compound is a pseudopeptide which uses a hydroxamic acid moiety to chelate the zinc and binds to a broad spectrum of MMP targets with nanomolar affinity. Batimastat was designed based on the glycine-leucine cleavage site in collagen that is proteolysed by MMPs -1, -8, and -13. It is configured in such a manner that the scissile bond of the peptidomimetic substrate cannot be hydrolyzed, forming an inactive enzyme/pseudosubstrate complex (5-8).

Despite promising preclinical results in which batimastat exhibited antitumor, antiangiogenic, and antimetastatic effects, dose-limiting musculoskeletal side effects were observed during clinical trials in the form of join stiffness, inflammation, and pain (Table 1). Further, orally administered batimastat is highly insoluble with low bioavailability (9). Thus, trials were soon cancelled in favor of an analogue of batimastat which was chemically modified to improve oral availability. This next generation compound, marimastat, also showed promise in pre-clinical studies and exhibits greater oral bioavailability. However, the musculoskeletal side effects observed during clinical trials were still significant and, in some cancer patients, debilitating (10). Further, although marimastat is more orally bioavailable than batimastat, plasma concentrations

Table 1. Summary of select MMP inhibitors discussed

Compound name/description	Domain (s) targeted	Notes
Batimastat	Catalytic (zinc chelator)	Broad spectrum peptidomimetic; cancelled in phase III clinical trials
Marimastat	Catalytic (zinc chelator)	Broad spectrum peptidomimetic; cancelled in phase III clinical trials
MMI-270	Catalytic (zinc chelator)	Broad spectrum small molecule; cancelled in phase I clinical trials
Tanomastat	Catalytic (zinc chelator)	Selective for MMP -2 ,-3, -8, -9, -13; small molecule; cancelled in phase III clinical trials
Chemically modified tetracyclines	Structural ions (catalytic and/or hemopexin domain)	Broad spectrum small molecule; approved with additional clinical testing ongoing
Peptides or small molecules binding at dimer interfaces	Hemopexin domain	Selective for the MMP designed against; do not affect catalytic activity but do disrupt downstream dimer-mediated effects (cell migration, metastasis, etc.)
MDI-301	N/A	Preclinically tested in a topical ointment for a diabetes model; selective for MMP -1, -2 and -9; stimulates a concomitant increase in type I procollagen abundance
Liposome/nanoparticle drug delivery systems	Variable	Can be used to confer selectivity for the MMP of interest; allows for encapsulation of cytotoxic agents to reduce off-target effects

still remained generally poor. This has been attributed to rapid metabolism of the hydroxamic acid group since this group is reduced in the liver (11). Compounding this, the hydroxamate moiety can easily be cleaved off, decreasing further the plasma concentration of the inhibitor and thus reducing potency (12). As a result, clinical investigation of marimastat also was ultimately terminated.

As the crystal structures were solved for MMPs bound to various inhibitors, structural information could be used to understand inhibitor binding modes, map the druggable space, and then leverage this information to screen for better therapeutics. Analyses of batimastat bound to MMPs has revealed that the P1' bulky, aromatic moieties of batimostat optimally and fully occupy the hydrophobic S1' specificity cavity (13). The S1' cavity is an exosite adjacent to the active site which confers specificity for substrate binding. The depth of this cavity can vary to be either shallow, intermediate, or deep depending on the MMP. The amino acid sequence of the S1' site is less similar among the MMPs than the sequences at the catalytic domain with different binding arrangements possible within the S1' cavity (12). This information provides insight to optimize MMP inhibitors to improve specificity.

3. SMALL MOLECULE INHIBITORS

As information about the chemistry, structure, and pathophysiology of MMPs became available, so came the ability to capitalize on tools used to biochemically or computationally screen drugs in a high throughput manner. Phenotypic screens, in which a large library of drugs are assayed to assess desired, functional changes were utilized (14) along with *in silico* docking assays. Hits identified during screening are later tested in various biochemical assays to validate each compound as a potential drug. The information collected along with molecular modeling programs then allow for optimization of the lead compounds to improve binding affinity, drug potency, and selectivity (15, 16). By this time, the contribution of MMPs to progression of a diverse spectrum of pathologies had also come to light. Aside from cancer, MMPs have been implicated in arthritis, pulmonary disorders (including emphysema and chronic obstructive pulmonary disorder), post-myocardial infarction tissue remodeling, and multiple inflammatory diseases.

The next generation of drugs to reach clinical trials included the use of small molecules identified in such high throughput screens. Typically small molecule drugs differ slightly from peptidomimetics in the manner by which they inhibit MMPs. Binding of these drugs to the active site induces a structural modification which renders the protease inactive (17). Additionally, moieties which chelate the catalytic zinc began to be diversified and/or elaborated on; these groups included hydroxamates, carboxylates, thiols, and phosphorous-based zinc binding groups (18). Despite that all of these moieties are reported to coordinate the catalytic zinc with a significant decrease in potency, (6, 8) these drugs tend to be more selective with greater oral bioavailability and improved pharmacokinetic profiles (19).

One of the first small molecule compounds to reach clinical trials, MMI270, is a synthetic hydroxamic acid derivative with a sulfo-succinic acid motif. This compound is more water soluble than its predecessors and exhibits greater oral bioavailability (20). A sulfur dioxide moiety forms key hydrogen bonds with the amides in the amino acids of the MMP's main chain. Also important are hydrophobic contacts within the S1' site, which increase the binding affinity of the drug for the protease in a similar manner to that described for the peptidomimetics (5). Although MMI270 reduced tumor burden in rat tumor models of breast and endometrial cancer and demonstrated antimetastatic and antiangiogenic effects (21), trials were cancelled

due to reactions to the drug manifesting in rashes and musculoskeletal toxicity (22).

When small molecule tanomastat made it to clinical trials the drug was especially of interest because it was the first MMP inhibitor not considered a broad spectrum inhibitor. This drug uses a carboxylate moiety to chelate the catalytic zinc, and was designed to incorporate a biphenyl segment to bind into deep S1' pockets in order to selectively target MMP-2, -3, -8, -9 and -13. The drug was tested in clinical trials for use in solid tumors, rheumatoid arthritis, and prevention of organ transplant rejection and reached phase III clinical trials (23). Although musculoskeletal side effects were not observed with this drug, hematological toxicities in platelets and hemoglobin were observed. Furthermore, the drug did not confer progression-free survival or overall survival and was thus cancelled (24).

Ultimately, all of the above drugs tested were terminated with dose-limiting side effects as one of the main reasons. In hindsight, we now know that broad spectrum inhibitors can negatively affect normal and necessary physiologic processes. When the first MMP inhibitors reached clinical trials, only three MMPs had been identified but were only poorly characterized (25). While homology of the MMP family at the catalytic domain is high, MMPs also share structural similarity with another class of proteases, called A Disintegrin and Metalloproteinase (ADAM) which itself consists of over a dozen members. Broad spectrum inhibitors may also inhibit ADAM function as well as some other zinc-coordinating enzymes. We also now appreciate that failure to achieve trial end points is partially the result of trials conducted in inappropriate patient populations. Indeed, the most convincing preclinical data was generated when drug delivery was initiated *in vivo* in early stages of tumor development yet clinical trials included patients with diseases of all stages (25-27). Because MMPs drive disease progression, MMP inhibitors may be more successful if use was limited to diseases still in the early stages or for preventative measures. Further, although many cancers *frequently* express certain MMPs, it is not a guarantee this is the case for every patient and thus it cannot be ruled out that the drug target was never present to begin with (26). It is of utmost importance therefore that these facts are taken into consideration during design of future clinical trials investigating MMP inhibitors in cancer.

Other small molecule MMP inhibitors were tested for uses other than in cancer, such as after ischemic events. MMPs, particularly MMP-1, are known to remodel the left ventricular wall after myocardial infarct; however such remodeling is the leading cause of congestive heart failure and is predictive of morbidity and mortality. Broad spectrum MMP inhibition in *in vivo* models decreased such remodeling and slightly (though non-significantly) decreased total collagen content in the left ventricle (28). Other studies with broad spectrum small molecule inhibitors demonstrated that MMP inhibition could also attenuate ventricular dilation in the early remodeling period (29). This led to the testing of a broad spectrum MMP inhibitor, PG-116800, in patients with low left ventricular fraction ejection post myocardial infarction. However, no significant benefit to the patient was found (30). Since those trials were conducted, it has been determined that post-MI, there is a coordinated spatio-temporal response pattern in MMP and TIMP expression during repair of the myocardium. After chronic thrombotic occlusion, increases in the levels of MMPs -1, -2, -3, -7, -8, -9, -12, -13, -14 coincide with an acute decrease in expression of all four TIMPs. Some of the remodeling that occurs post-MI is necessary to reform proper cardiac structures and functions. The timing of the changes in expression are also of interest, as it has been shown that expression levels of MMPs differ greatly even months past the event (31). Because this MMP response post-MI is such a delicate, orchestrated response with a complex temporal profile, it is more than likely that benefits gained from the MMP inhibitor were overshadowed due to inhibition of MMPs conferring a positive response and/or inappropriate temporal administration of the drug.

4. CHEMICALLY MODIFIED TETRACYCLINES & NATURAL PRODUCTS

Tetracyclines are natural products produced by *Streptomyces* originally identified in the 1940s which were initially characterized as having bacteriostatic activity and are still commonly prescribed for use as antibiotics. Over the course of their long history tetracyclines and their analogues were also observed to inhibit the activity of MMPs 1, 2, 8, 9 and 13. The value of this observed MMP inhibition (which is unrelated to its antibacterial activities) eventually came to be appreciated (32, 33). While the exact mechanism of inhibition remains enigmatic, models suggest that, similar to the previous classes of drugs, tetracyclines and their analogues coordinate the catalytic zinc and occupy the S1' specificity exosite (34). Research also indicates these compounds may interact with the structural zinc and/or calcium atoms in either the catalytic domain or the hemopexin domain to destabilize the MMP's tertiary structure, thereby preventing catalysis (35). Not only are tetracyclines easily and cost effectively isolated and have been used safely as antibiotics for decades, their toxicity and efficacy profiles and pharmacokinetic properties are well described (36). Indeed, the single FDA-approved MMP inhibitor to date is doxycycline, a chemically modified tetracycline which is used to treat periodontal disease. Periodontitis is characterized by inflamed gingival tissue with significantly elevated levels of MMPs, particularly collagenases, which originate primarily from infiltrating leukocytes (37). The orally administered drug is marketed as Periostat® and is provided in capsule or tablet form at doses below the concentration necessary for antibiotic effects to be observed.

Because of the reasons described above, tetracyclines and their chemically modified analogues have been and are being investigated for other indications as an MMP inhibitor. These include, but are not limited to, investigating the use of doxycycline in non-Hodgkin Lymphomas, to decrease reperfusion injury or ventricular modeling after cardiac events, to enhance vascular wall stability after hemorrhage in the brain, polycystic ovarian syndrome (PCOS), type II diabetes, chronic obstructive pulmonary disease (COPD), cystic fibrosis, multiple sclerosis, and others. The information that is currently available for these trials indicate moderate success with the intent to continue clinical testing. Intent has also been made to test these drugs in combination with chemotherapeutics in patients with various cancers. Of significance, to date no severe adverse reactions have been clinically observed and available trial results generally report improvements in patient health and disease presentation.

What we have learned from the success of tetracyclines and the promise they offer our future is that through attention to detail and perhaps some serendipity, old drugs and common natural products may be readily repurposed for new indications. In fact, the use of many compounds derived from common foods is under investigation for potential MMP inhibition. Curcumin, a component of the South Asian spice turmeric, has been shown to decrease MMP-2, -9, and -14 expression in various cancers, thus leading to decreased MMP activity and decreased cancer cell migration and invasion (38-40). Similarly, antioxidant polyphenols in common foods, such as resveratrol and quercetin from grapes and wine and oleuropein and hydroxytyrosol from olive oil have also been shown to decrease MMP expression and activity to decrease cancer cell migration, invasion, and angiogenesis (41-44). Clinical trials testing these compounds or their analogues have been conducted in cancer patients; however the testing of these compounds in patients is still in the early stages despite years of research. Though data collected thus far is encouraging, long term benefits and studies involving comparators or synergistic effects of such supplements with standard care have yet to be thoroughly investigated. Further, it is difficult, if not impossible, to patent such compounds. Because of this, pharmaceutical and industrial interest and investment as yet is insufficient to sponsor large clinical trials or file for approval of these compounds as therapeutic agents.

5. INNOVATION IN HIGH THROUGHPUT *IN SILICO* PROGRAMS FOR IDENTIFICATION OF POTENTIAL THERAPEUTICS

At the time of the earliest clinical trials for MMP inhibitors, the sheer size of the MMP family was not yet fully realized. Compounding this was the fact that the surface had only been scratched with regard to understanding the function of MMPs in normal, healthy tissues. It is now understood that the degradome of a particular MMP may overlap with the degradadome of another MMP. Because certain MMPs can have protective functions in certain tissues or pathologies, broad spectrum inhibition may result in no significant benefit to patients, adverse effects, and potentially even driving disease progression through inhibition of these protective MMPs (45). Even the temporally coordinated expression patterns of MMPs influence disease status. Following myocardial infarction, neutrophil infiltration is followed by macrophage infiltration into the damaged tissue. This leads to early release of MMP-9 from neutrophils and then subsequent release of MMP-2 from macrophages. Initially, this leads to a clearing of necrotic tissue, but accumulation of these proteinases can lead to cardiac rupture or death soon after the initial event (46). As research progresses, technology advances, and old paradigms are challenged, the reasons why clinical trials for MMP inhibitors have largely failed become clear while the need for development of highly selective inhibitors which can access their targets appropriately (both physiologically and temporally speaking) have been underscored. Efforts to meet this demand have engendered innovation in the form of technological approaches to drug screening, drug design targeting unique exosites or domains, and development of specialized drug delivery systems in the post-trial era.

Such innovation now includes complex and advanced *in silico* evaluation algorithms, drugs which bind to the catalytic domain in a non-zinc-chelating manner, drugs which inhibit allosterically, and/or drugs which are novel for their methods of delivery. Structure-based methods like those discussed above have been drastically improved as information and advanced bioinformatic tools became available. One example of such technological advancement is the ability of many docking programs to predict if a chemical compound has the potential to be orally bioavailable. Lipinski's rule of five was developed by Christopher Lipinski after he observed that most drugs which can be administered orally are small molecules which are moderately lipophilic. There are four rules built around multiples of 5 which are designed to decrease the attrition rate of drugs in clinical trials. The rules are as follows: (i) there must be no more than five hydrogen bond donors; (ii) there must be no more than ten hydrogen bond acceptors; (iii) the molecular mass must be less than 500 daltons; (iv) the partition coefficient log P must be no more than 5.0. Algorithms have been built for drug design which limit hits to those falling within these guidelines in an effort to decrease failure in the clinic as a result of unacceptable pharmacodynamics (47).

MMP-13 is responsible for the collagen break down in joints that is characteristic of both rheumatoid arthritis (RA) and osteoarthritis (OA). Analyses of co-crystal structures of MMP-13 to identify pharmacophore

and substitution patterns allowed for design of drug scaffolds which were capable of designing selective and high affinity inhibitors. The scaffold compounds designed for MMP-13 were then also docked *in silico* into MMPs -2, -12 and -14 to confirm selectivity for MMP-13. From this exercise a selective pthalomide scaffold was identified which forms hydrogen bonds with the backbone of the amino acid chain of MMP-13 and pi-pi interactions with the histidine which coordinates the active zinc. This compound however does not chelate the catalytic zinc. To improve the potency of this pthalomide scaffold, MMP-13 structural water molecules were then analyzed for their potential contribution as binding partners with a drug. Optimizations to the scaffold were then made that could leverage binding with these water molecules in order to improve binding affinities. After several rounds of such optimization, a compound was identified which, *in silico*, bound selectively to MMP-13 at the S1' site, interacted with the catalytic zinc via a structural water intermediate, and formed several other important hydrogen bonds with structural water molecules that occupy space when the protein is in solution. This method of design yielded a drug which exhibited nanomolar binding affinity *in vitro* and was demonstrated to be highly selective for MMP-13 (48).

Rheumatoid arthritis is characterized both by chronic inflammation of synovial tissue and degradation of collagen in joints. MMP-8 is secreted by neutrophils and is overexpressed in RA. MMP-8 is responsible for driving progression of this disease through its collagenolytic activity as well as its ability to regulate mediators of inflammation by processing certain chemokines (49). *In silico* screening of a curated compound library for compounds which exhibit selectivity for the deep, hydrophobic S1' exosite of MMP-8 was recently performed. In order to circumvent off-target binding, drugs which chelated the catalytic zinc were excluded from the hit list as were drugs which were not predicted by the program to pass Lipinski's five ADME requirements. As above, the contribution of water molecules was also considered when determining binding affinities. This study was especially interesting because molecular dynamics simulations and free energy calculations were carried out during the screening process to ensure a best-fit scenario mimicking protein behavior in solution. The structure-based pharmacophore model developed in this study was a compound that has two hydrogen bond acceptors and one donor with an aromatic, a hydrophobic aromatic ring, does not chelate the zinc, meets standard ADME criteria, and considers the dynamic motion of the target protein in solution (50).

Fragment based drug design (FBDD) evolved as a means to overcome some of the limitations associated with high throughput *in silico* screening tools. High throughput screening requires a huge number of compounds which are docked into only a small region of druggable chemical space. Thus, the hits identified tend to be few in number and are comprised of hydrophobic and high molecular weight ligands which are difficult to optimize (51). FBDD involves identification of small chemical compounds or discrete functional groups, referred to as "fragments", from a much smaller library which bind to the drug target even with weak to modest affinity. A collection of these fragments which bind to various regions of the target site are then mapped out and incorporated into one compound through a linker, yielding a drug whose binding affinity and selectivity is greater than the fragments. Ideally, the final, optimized compound complements the electrostatic map of the target site and exploits pre-existing hydrogen bonds or van der Waal interactions to yield a highly selective, potent drug with high affinity for the target (16, 51, 52). Recently, Durrant *et al* used FBDD in conjunction with molecular dynamic studies to design a highly potent drug capable of binding multiple sites of MMP-2. The algorithm used to perform these studies accounts for target flexibility as well as changes in conformation induced by binding of the drug. This novel virtual screening approach is useful especially for targets like MMPs, whose binding pockets are highly flexible, making predicting binding affinity *in silico* by traditional methods difficult and unreliable. Previous molecular dynamics simulations of MMP-2 indicate that the unbound catalytic domain oscillates between an "open" (rare) and "closed" (more frequent) state, with the open state adopting a tunnel-like shape in the S1' pocket (53). This information was then leveraged by Durrant *et al* to design an inhibitor which may bind to MMP-2 with high affinity, high potency, and high selectivity. 3-hydroxy-2-methyl-4-pyrone (maltol) was chosen as the zinc binding group as this was identified by a previous group as having increased selectivity and potency compared to the hydroxamate-containing predecessors (54). Further, this maltol group is oriented in a manner that it can easily be extended with fragments extending into multiple exosites on MMP-2. Small chemical groups were then docked into either the large, flexible, hydrophobic S1' specificity pocket as well or the solvent-exposed and highly flexible S2'/S3' pockets. The docking studies then utilized snapshots of the MMP in its natural various conformations identified in the earlier molecular dynamics study as well as conformations adopted as a result of the induced fit of the ligand. Average binding affinity was reported as the readout. Interestingly, comparison of the scores determined for different conformational states of the protein for some compounds were vastly different, indicating that protein flexibility and conformations induced by the inhibitor can have a major impact on binding. From the fragments identified in this study, composite compounds were generated linking the fragments predicted to bind in S1' and fragments predicted to bind to S2'/S3' together with maltol. *In silico* docking studies of the composite molecule were then performed. Combination of these fragments with the identified zinc binding group improved predicted potency and exhibited higher predicted binding

energy (55). Although *in vitro* experiments have yet to be conducted, the possibilities presented by this type of method could change how we approach drug design for any target.

6. ALLOSTERIC INHIBITORS: THINKING OUTSIDE THE CATALYTIC SITE

For many years, drug discovery and development focused on inhibitors that bind in the MMP active site and chelate the catalytic zinc. However, due to the high similarity between the catalytic domains of MMPs, which are also highly homologous to the catalytic domains of ADAMs, the prospect that other domains could be targeted became attractive in the post-clinical trial era. The hemopexin-like domains of MMPs are made up of four structurally similar hemopexin-like repeats. Each of these segments forms a blade shape produced by four antiparallel β-strands and together the four blades form a propeller-like structure (56, 57). The inner three strands of the blades share the highest homology within the MMP family whereas the outer β-4 strand shares the least. Because of this dissimilarity, these domains can be targeted to increase drug selectivity even for MMPs such as the gelatinases which are closely related and for which the catalytic domains are nearly identical (56). The propeller funnels into a tunnel structure which is highly solvated and coordinates as many as four ions in a linear manner. These ions are thought to stabilize the structure of the hemopexin-like domain (57). Additional structural stability is provided by a disulfide bridge which forms between two cysteine residues on blades I and IV (58). The hemopexin domain has different roles depending on the MMP in question; for MMP-14, it enables dimerization necessary for activation of MMP-2 and for cell migration (59, 60), MMPs -2, -9 and -13 are regulated by tissue inhibitors of MMPs (TIMPS) at this domain (61), and in the case of MMP-1 substrate specificity is conferred by participation in cooperative binding to the triple helical collagen (62-64). Dimerization at the hemopexin-like domain serves many functions. Because of this, targeting effectors of dimerization at the hemopexin domain may be more effective than actually attenuating catalysis.

Studies have shown that MMP-9 can homodimerize and can heterodimerize with cell surface molecule CD44; either of these types of dimers can increase cell migration. Downstream effects can lead to cytoskeletal rearrangements necessary for cell migration, assembly of invasion machinery, and stimulation of angiogenesis (58). Prevention of dimerization significantly decreases tumor size, MMP-mediated cell invasion through the basement membrane, angiogenesis, and metastasis of tumor cells both *in vitro* and *in vivo* in mouse xenograft models animal models (65, 66). Studies using short peptides which were designed to mimic the motifs in the outermost strands of the MMP-9 hemopexin domain which bind at the dimer interface have been performed. These structure-based inhibitory peptides successfully inhibited cancer cell migration *in vitro* (66). This same group successfully performed similar inhibition studies both *in vitro* and *in vivo* using peptides mimicking the amino acids which allow for membrane-bound MMP-14 homodimerization and heterodimerization with CD44, which also leads to cancer cell migration, invasion, and metastasis. These peptides prevented MMP-14-mediated cancer cell migration *in vitro* and significantly reduced the number and size of metastases in a mouse model using MDA-MB-435 xenografts (67). Developing drugs which delay or prevent metastasis by keeping tumors contained until they can be surgically removed may therefore be even more important than development of drugs which reduce tumor growth. Short peptides as described herein are useful in that they are generally very selective, highly potent, and have lower toxicity with less accumulation in tissues. Although peptides as drugs have some advantages and were successful in these pilot studies, peptides on their own as drugs are not always ideal. This is a result of their poor membrane permeability coupled with low oral bioavailability, requiring they be injected directly into the target tissue, and rapid clearance from the body (68).

Virtual screening of a library of commercially available small molecule compounds which may bind to the hemopexin domain of MMP-9 has also yielded identification of a compound which decreases cancer cell migration *in vitro* and metastasis *in vivo*. Although this compound did not affect cell viability, MMP-9 expression levels, or MMP-9 proteolytic activity, it significantly reduced tumor cell proliferation and decreased the number and volume of metastases (65). This study, in agreement with those discussed above, strongly support the use of inhibitors targeting the hemopexin domain in conjunction with conventional cytotoxic drugs to reduce tumor burden and block metastasis. *In silico* analysis of the MMP-14 hemopexin-like domain indicates a druggable pocket-like space in the center of the structure. Docking studies predict that small molecule inhibitors which can bind in this space may allosterically block dimerization. These studies identified a compound which is selective for MMP-14 versus MMP-2, was not cytotoxic, and did not affect catalytic activities. This compound was effective in significantly decreasing cancer cell migration *in vitro.* Furthermore, *in vivo* studies resulted in decreased tumor size and a fibrotic tumor phenotype due to decreased cancer cell invasion (69).

While important strides have been made demonstrating the potential of targeting the hemopexin domain with therapeutics, recent work further highlights how the coveted specificity can be achieved at this domain although such drugs have not yet been designed. As alluded to briefly above, MMP-1 is a rather unique MMP in that the hemopexin domain and linker region provide some measure of substrate specificity for triple helical collagen (70). Triple helical collagen is made up of three intertwined

strands. Each collagen strand is a polyproline type II helical chain with repeating Gly-X-Y triplets, where X and Y are typically proline and hydroxyproline, respectively. Three of these chains then intertwine to form triple helical collagen (71). MMP-1 has been shown to be overexpressed by the synovial cells of joints in rheumatoid arthritis and osteoarthritis and has been shown to contribute to the pathological progression of these diseases. When triple helical collagen binds to MMP-1, the P1' of the substrate interacts with the S1' exosite at the catalytic domain. Sequence alignment studies using bioinformatic tools identified key leucine residues conserved at P1' and P10' in each of the three alpha strands of collagen. Interestingly, it was then shown that P10' must act cooperatively with the hemopexin domain in order for the collagenolytic activity of MMP-1 to occur. A hydrophobic pocket at the S10' exosite, which is located at MMP-1's hemopexin domain. forms hydrophobic interactions with the P10' leucine residue. This interaction is required for proper orientation of the substrate in order for collagenolysis to occur (62). This study marks the S10' subsite at the hemopexin domain as a completely unique targeting site for the treatment of OA and RA.

Another domain with high potential for design of drugs achieving high selectivity is the prodomain. MMPs are synthesized as zymogens; with the exception of some membrane-type MMPs they are secreted as inactive precursors and require proteolytic cleavage of the prodomain in order to become active. The prodomain consists of approximately 80–90 amino acids. A key cysteine residue interacts with the catalytic zinc atom through its thiol group, keeping the propeptide in a cap-like formation and effectively blocking the active site to render the MMP inactive. Stepwise proteolysis of the prodomain disrupts coordination of the cysteine thiol and catalytic zinc ion, exposing the catalytic domain and allowing the MMP to become fully active (72). The propeptide domains, with the exception of a conserved 8 amino acid sequence, have significantly less sequence similarity compared to the catalytic domains. Targeting these domains to block cleavage and thus MMP activation is therefore a viable option. Although preclinical experiments inhibiting the activators of MMPs have been completed with success, inhibitors binding directly to this domain have yet to be investigated. Monoclonal antibodies may be useful, as they may either sterically block the cleavage site or initiate mechanisms leading to antibody-dependent proteolysis of the recognized target. Antibodies have high bioavailability, long half-lives, and ideal membrane permeability and tissue distribution and therefore are ideal for therapeutic use (73).

7. DECREASING SYSTEM-WIDE EXPOSURE TO INCREASE SELECTIVITY/DECREASE ADVERSE EVENTS

One of the largest challenges faced for development of MMP inhibitors is how to selectively deliver the drug only to where it is needed. Systemic exposure of broad spectrum inhibitors was the primary reason for the serious adverse effects observed in early clinical trials. To circumvent the issue, several innovative ways to deliver the drug only to the region of interest have been investigated.

For many patients suffering from diabetes mellitus, foot ulcerations are a serious complication affecting an estimated 10-25% of patients. Although MMPs normally are beneficial in wound healing, remodeling the extracellular matrix during re-epithelialization and allowing for vascularization, overexpression of MMPs (particularly but not limited to MMPs -1, -2, -8, -9 and -13) in diabetes leads to inappropriate and excessive degradation of epithelial tissue. Further, MMPs promote activation of bioactive signaling molecules causing a positive feedback loop which drives inflammation (74). Retinoids are compounds with biological activities similar to their parent compound, vitamin A. Retinoids can cross the cell membrane, enter the nucleus, and bind to retinoid response elements on DNA to regulate expression of certain genes. In this manner, retinoids can decrease expression of collagenase MMP-1, increase expression of type I procollagen, and increase pathways necessary for repair of damaged epithelia (75). Two retinoid-based compounds are approved for use in the U.S. for photodamaged skin, acne, acute promyelocytic leukemia, and keratosis pilaris; however, these drugs are not considered MMP inhibitors but were approved for their use to induce promyelocyte differentiation (in leukemia) and increase total collagen content. However, retinoic acid-derived compounds, which can only be administered topically, are associated with photosensitivity and irritation (76). Recently, a synthetic retinoid referred to as MDI 301 which can inhibit MMP activity and simultaneously stimulate collagen production was identified and formulated as a topical ointment for potential use in treating skin ulcerations associated with diabetes mellitus. When administered to skin biopsies of diabetic patients, this drug reduced the activities of MMPs -1, -2 and -9 with a concomitant increase in type I procollagen abundance. Further, the ointment improved the mean structural deficit score, which is a measure of collagen structure and organization. *In vivo* studies with this drug have indicated it can increase wound repair time and is also less irritating to skin than parent compound retinoic acid (77-79). As diabetic foot ulcerations are the leading cause of non-traumatic amputations, development of such an agent is critical for treating an ever growing population suffering from this disease.

As has been discussed in the preceding section of this chapter, osteoarthritis is a debilitating joint disease characterized by the breakdown of the collagen in joints. Available treatments however are merely palliative. One of the biggest challenges for any drug tested for this disease is access; the cartilage found in joints

is not vascularized and thus diffusion of drugs out of capillaries and into articular cartilage is virtually nil. Thus, in order for an inhibitor to reach its target, it must either be systemically administered at high concentrations to reach the therapeutic dose or delivered intra-articularly. A study using a selective MMP-13 inhibitor with low solubility was recently performed in which the drug was injected directly into the joints of rats. Approximately two months after the injection, low levels of the drug were still found in the cartilage but throughout the study the drug concentration in the plasma remained below the detection limit. Additionally, there was no difference between the drug- or control-treated group in toxicity or in limb use. Assessment of MMP-mediated collagen degradation in bovine articular cartilage explants indicated significantly less activity in the drug-treated samples, with an IC50 of just 20nM. In an *in vivo* animal model, injection of the inhibitor into the cartilage of rat joints inhibited 100% of MMP-13-induced collagen degradation even 21 days past injection (19). This study validates intra-articular administration of a selective MMP-13 inhibitor as a promising, long-term therapeutic option that will target the pathology of osteoarthritis at its root. Localized delivery will effectively alleviate the adverse effects previously observed in clinical trials of drugs with broad spectrum, systemic distribution.

8. INNOVATIVE DRUG DELIVERY SYSTEMS DECREASE NON-SPECIFIC BINDING

The majority of the chemotherapeutic agents currently on the market are effective against any replicating cell, system-wide. Such approaches damage healthy cells and cause the side effects typically associated with cancer treatment such as hair loss, nausea or diarrhea, or blood disorders among others. Because the early, unsuccessful MMP inhibitors were also non-specific and systemically available, recent approaches include design of drug delivery systems that are only effective within the tumor environment. Several monoclonal antibodies have been clinically tested in cancer patients for use in combination with standard chemotherapeutics. However, patients still suffer from the side effects of chemotherapy and significant improvement in quality of life or patient prognosis was not conferred. In an attempt to eliminate the need for general chemotherapy altogether, antibody-conjugated drug delivery systems are being investigated. Such systems are ideal because they leverage the high selectivity of monoclonal antibodies to deliver highly cytotoxic drugs. These systems typically incorporate a flexible linker to covalently conjugate the antibody with the drug; these linkers are of significance because they must keep the system stable in circulation yet facilitate drug release following internalization by the cells within the target tissue (80). Recently, a liposomal drug delivery system was designed which incorporated a tumor cell-specific antinucleosome monoclonal antibody (termed mAb 2C5) linked to cell penetrating TAT peptide (TATp) via a MMP-2 cleavable peptide. In this model, the antibody would target the system to tumor cells by binding directly to the cell-surface antigen. Cleavage of the peptide by cancer cell-secreted MMP-2 would then expose the TATp moiety anchored in the surface of the liposome. TATp then initiates efficient translocation of the liposome across the cell membrane (81). While this proof-of-concept study did not seek to incorporate a cytotoxic or therapeutic agent into the inner core of the liposome, multiple studies have successfully delivered such compounds using liposomes as delivery vessels (82). However, the use of an MMP-cleavable peptide along with a tumor cell-specific antibody incorporated to such a system confers selectivity and should prevent the off-target effects typically experienced by traditional approaches.

Similar to the above approach, nanoparticles made up of mesoporous silica have recently been used to deliver a cytotoxic agent selectively to tumor cells. The system was designed so that polyanions adhere to the surface in order to block nonspecific particle uptake under physiological conditions. The polyanions were linked to the nanoparticle system via the MMP core substrate peptide sequence PLGVR; this peptide acts as a “trigger release” because cleavage at this peptide by MMPs secreted by cancerous tissues releases the polyanions so that adjacent cells will then take up the particles. The MMP-cleavable peptide together with an RGD motif confer selectivity to the system, as most human cancers overexpress both MMPs and RGD receptors. Following cleavage of the MMP substrate and then recognition of the newly exposed RGD domain endocytosis occurs. Cytotoxicity is conferred by the chemotherapeutic agent doxorubicin hydrochloride, which is efficiently incorporated into the hydrophobic core of the multifunctional envelope. Once the nanoparticles are endocytosed the drug is released into the cell and causes cell death. *In vitro* experiments using these nanoparticles indicate the system selectively crosses the membrane only of cells which express the MMPs to cause cytotoxicity, as cleavage of the MMP substrate sequence is required to release the cell-repelling polyanions (83).

Chitosan is a linear polysaccharide which is protonated when dissolved in solutions of neutral pH. This material has been found to be capable of spontaneously forming nanoparticles when combined with nucleic acid due to its negative charge (84). Recently, in a novel approach to genetic medicine, MMPs -3 and -13 were silenced in chondrocytes in an attempt to reduce their dedifferentiation for potential use in autologous chondrocyte implementation (ACI) (85). ACI is a biomedical technique performed in patients who have suffered extensive damage to the cartilage in joints; surgical implementation of chondrocytes can initiate collagen regrowth and revascularization in the affected area. However, in some patients these chondrocytes will dedifferentiate into fibroblast-like cells after graft implant,

leading to graft failure, separation of the implanted cells, and arthrofibrosis among other symptoms. This dedifferentiation and its associated symptoms has been shown to be potentiated by secretion of MMPs -3 and -13 by the fibroblast-like cells (86, 87). Zhao *et al* experimentally showed *in vitro* that a chitosan/MMP shRNA nanoparticle delivery system was able to retain and protect from degradation the condensed plasmid DNA. Furthermore MMP expression by chondrocyte cells was effectively repressed and these cells proliferated *in vitro* with a lesser rate of dedifferentiation (85). Whether these transfected cells can reduce the MMP-mediated failure of such grafts in an *in vivo* model however remains to be seen.

In order to increase patient compliance, the preferred method of drug delivery has traditionally been in the oral form. While preferred over daily injections or invasive delivery methods, the burden of orally taking multiple large tablets and (in some cases) multiple dosages per day is also not ideal. In some individuals the binders and excipient materials used in manufacturing can irritate the digestive system or leave unpleasant aftertastes when consumed in large amounts. Recently, a novel system was designed which utilizes an injectable MMP-degradable hydrogel capable of delivering recombinant tissue inhibitor of MMP 3 (rTIMP-3) following myocardial infarction to attenuate adverse left ventricular remodeling (88). The hydrogel is a polysaccharide meshwork whose crosslinks can be degraded by multiple MMPs to liberate rTIMP-3. This macromolecule then locally inhibits the proteolytic activities of the MMPs responsible both for degradation of the ECM as well as cleavage of bioactive signaling molecules. In a porcine *in vivo* model of MI the hydrogels were nearly completely degraded two weeks after injection in the damaged myocardium but there was no significant change in volume of the healthy tissue, indicating the gel is responsive only in a MMP-secreting pathological model. Further, delivery of rTIMP-3 significantly attenuated MMP activity within the interstitial MI region but yet no decrease in activity was observed in remote, non-MI myocardium tissue. 14 days after treatment with the rTIMP-3 hydrogel, animals exhibited improved left ventricle ejection fraction, a reduction in chamber dilation, and an attenuation of the pulmonary capillary wedge pressure compared to sham-injected animals. Echocardiogram assessments 28 days after treatment showed that these benefits were maintained (88). Although more work will need to be done to evaluate recoveries over an extended timeframe and to assess recurrence risks, this proof-of-concept study in a large animal model is certainly promising and warrants additional investigation.

9. FUTURE DIRECTIONS

The failure of the early MMP inhibitors in clinical trials is a stark reminder of why careful attention must be given during the development process to drugs that exhibit high selectivity and affinity for their target. Furthermore, perspectives on how clinical trials are designed in order to maximize impact need to be aligned with the end goal. For example, if an inhibitor is designed to decrease or prevent metastasis the patient population should consist only of early stage cancer patients. To achieve clinical success, drugs must be innovative in either how they inhibit MMPs, how selectivity is achieved, how they are administered to the patient, or a combination of these attributes. Although clinical success thus far with inhibitors of MMPs has been limited, because these proteinases directly contribute to disease progression they remain a viable and desirable target.

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