Matrix Metalloproteinases and Colon Anastomosis Repair: A New Indication for Pharmacological Inhibition?

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Abstract: Excessive matrix metalloproteinase activities have been implicated in the pathogenesis of intestinal anastomotic dehiscence, a serious and potentially life-threatening complication following gastrointestinal surgery. In this review, the properties of matrix metalloproteinases are summarized followed by presentation of clinical therapeutic interventions with synthetic matrix metalloproteinase inhibitors and novel experimental data on colon anastomosis repair that warrant exploration of these drugs in surgical colorectal patients.

Keywords: Proteinases; Wound healing; Colorectal carcinoma; Colorectal surgery; Anastomosis; Collagenolysis; Collagen; Hydroxamate inhibitors.

INTRODUCTION

Proteinases are involved in many different and fundamental biological processes such as coagulation, immunity, inflammation, angiogenesis and tissue remodeling to name but a few. In addition, proteinases have been implicated in the etiology of diseases such as cancer, cardiovascular diseases and Alzheimer's disease.

There are four classes of mammalian proteinases: (1) aspartic proteinases, (2) cysteine proteinases, (3) metalloproteinases, including the matrix metalloproteinases (MMPs), and (4) serine proteinases, that require an aspartate residue, a cysteine residue, zinc, and a serine residue, respectively, for activity. The proteinases most pertinent to tissue repair processes are the serine proteinases and the MMPs.

In this review the biochemical properties and biological roles of MMPs will first be summarized: this excludes other metalloproteinases, such as ADAMs (a disintegrin and metalloproteinase domain), ADAMTSs (a disintegrin and metalloproteinase thrombospondin domain), astacins, and neprilysins. Following the general summation of MMPs, results of the many therapeutic clinical trials with synthetic MMP inhibitors (MMPI) in oncology are presented. Then colorectal cancer occurrence, surgical treatment and complications are reviewed. The chapter is concluded by going over the experimental basis for MMPI therapy in anastomotic repair after surgical colorectal resection.

MATRIX METALLOPROTEINASES (MMPS)

MMPs are a family of zinc-dependent endopeptidases with 23 human members known to date [1]. The interstitial collagenase-1, -2, and -3 (MMP-1, MMP-8, and MMP-13), gelatinase A and B (MMP-2 and MMP-9), stromelysin-1, -2, and -3 (MMP-3, MMP-10, and MMP-11), macrophage elastase (MMP-12), matrilysin-1 and -2 (MMP-7 and MMP-26), epilysin (MMP-28), and membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24, and MMP-25) are the main vertebrate MMPs.

Structurally, all MMPs contain a N-terminal propeptide and a catalytic domain. MMP-2 and MMP-9 have three repeats of fibronectin type II domain inserted in the catalytic domain. A C-terminal hemopexin-like domain is common for all MMPs except for MMP-7. Membrane-type MMPs have a C-terminal membrane-anchored domain [2]. Substrate specificities, chromosomal locations and 3D structures are available at http://www.circresaha.org as data supplement to Visse and Nagase [1]. For more details reader should refer to other reviews on MMPs published in books and in medical journals [1-4].

MMPs are induced at the transcriptional level by pro-inflammatory cytokines, growth factors, hormones, ultraviolet radiation, physical stress and also by cell-cell contacts and cell-matrix interactions via integrins [2]. Retinoids and glucocorticoids, on the other hand, generally down-regulate MMP gene expression although retinoic acid under certain circumstances induces MMP-13 [5].

MMPs are secreted into the extracellular environment as zymogens or proMMPs on demand, except in the case of neutrophils, macrophages, and Paneth's cells where MMPs are stored in granules. Latency is maintained via linkage between cysteine in the propeptide domain and the zinc ion at the catalytic site [6]. After secretion, proMMPs are positioned at specific extracellular sites. Amongst the most important MMP regulators are the MMP-binding molecules of the extracellular environment (Table 1). These dock the MMPs at specific sites of the cell membrane or the extracellular matrix (ECM), and provide MMPs with a specific orientation towards their substrate or activator. Although not entirely delineated, MMP activation in tissues most likely involves proteolytic cleavage of the propeptide domain by tissue or plasma proteinases in a stepwise fashion [42]. The membrane-associated MMP-14 is unique in that it is activated in the cell by the serine proteinase furin and can activate MMP-2 at the cell surface via formation of a

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Table 1. Association of MMPs with Extracellular Components Belonging to the Plasma Membrane or to the ECM

MMP domain	Plasma membrane	ECM
MMP-1		Cartilage matrix [7]
MMP-1	Cancer cell membrane [8]	
MMP-1	Osteoclastoma cell membrane [9]	
MMP-1	Cancer cell membrane via EMPRIN¶ [10]	
MMP-1	α2 integrin [11]	
MMP-2		Endothelial cell-generated ECM [12]
MMP-2	Osteoclastoma cell membrane [13]	
MMP-2	Invadopodia and lamellipodia [14]	
MMP-2 F*		Denatured collagen [15]
MMP-2 F*	β1 integrin·collagen I complex [16]	
MMP-2 H*	αvβ3 integrin [17, 18]	
MMP-2 H*	MT1-MMP·TIMP-2 complex [19]	
MMP-2 H*		Fibronectin [20]
MMP-3		Osteoid [21]
MMP-3 H*		Native collagen I [22]
MMP-7	Heparan sulfate proteoglycan [23]	Heparan sulfate proteoglycan [23]
MMP-9	α2(IV) chain of collagen IV [24]	
MMP-9	CD44 [25, 26]	
MMP-9	Plasma membrane [27-29]	
MMP-9	Lamellipodia of cell membrane [30]	
MMP-9		Native collagen I [28]
MMP-9		Cartilage matrix [31]
MMP-9	β1 integrin at focal contacts [32]	
MMP-13		Native collagen I [33]
MMP-13		Subosteoclastic bone matrix [34]
MMP-13	uPARAP§ [35]	
MMP-13 H*		Native collagen I [36]
MMP-14	Podosomes/invadopodia [37, 38]	
MMP-14	Claudin-1 at cell-cell contacts [39]	
MMP-14, MMP-15, MMP-16, MMP-24	Transmembrane [4]	
MMP-17, MMP-25	Glycosyl-phosphatidyl inositol-anchored [40, 41]	

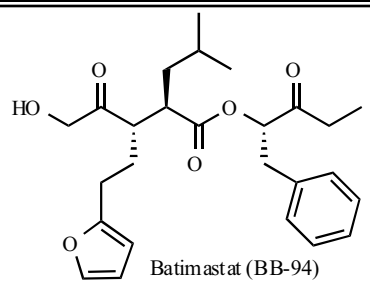
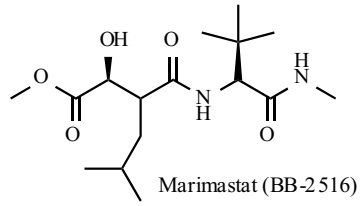
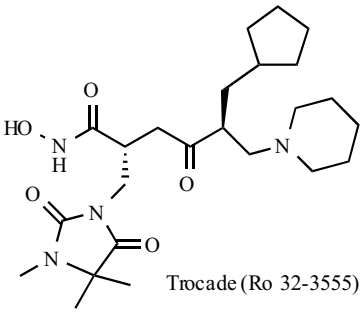
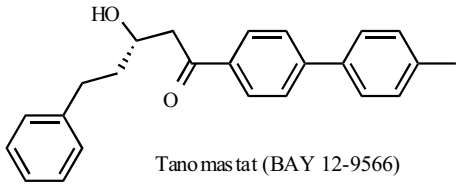
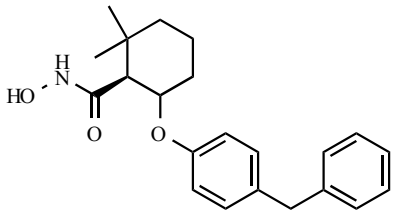
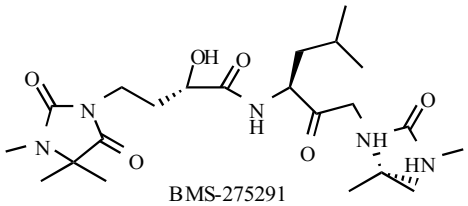
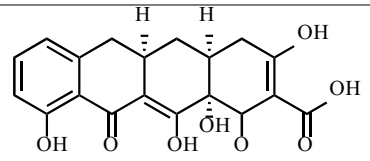
*H: hemopexin-like domain; F: fibronectin-like domain. ¶Extracellular matrix metalloproteinase inducer.
§uPARAP: urokinase plasminogen activator receptor-associated protein.

trimolecular complex with tissue inhibitors of metalloproteinases-2 (TIMP-2).

It has also been shown that matrix constituents can be involved in the activation of MMPs. For example, contact of cancer cells with type I collagen results in MT1-MMP and MMP-2 activation, and collagenolysis. Moreover, α2 integrin on keratinocytes can infer catalytic activity of MMP-1 even without concomitant loss of the propeptide domain [43]. There are indications that these activations require redistribution-clustering of membrane proteinases and proteins, which involves integrins as well as cytoskeletal reorganization [13, 29, 39, 44]. More mechanistic studies are required to elucidate this posttranslational regulation of extracellular proteolysis. The critical event is the establishment of a contact between different proteinases that can activate each other, which then leads to high proteolytic activities against a specific substrate at specific points of the cell membrane. These contact points correspond to functional domains of the membrane, such as invadopodia or podosomes of cancer cells [14, 37, 38, 45] or lamellipodia at the leading edge of normal cells [30, 38]. It may be speculated that if MMPs are overexpressed in pathological situations for instance, their physiological binding sites may become saturated, which may favor their interaction with ECM components that are not their normal substrates. It is also of interest that MMP-1, MMP-2, MMP-3, and MMP-9 are found in circulating blood [46]. The biological meaning is unknown but nonetheless supports the view that some MMPs may act at sites remote from their cellular origin.

Another line of control of the action of MMPs is that exerted by the naturally occurring α2-macroglobulin and TIMPs, which exist in four subtypes (TIMP-1, TIMP-2, TIMP-3, and TIMP-4). TIMPs bind to activated MMPs with high affinity in an equimolar ratio.

Table 2. Non-exhaustive List of Small-Molecule MMP Inhibitors (MMPI) in Cancer Clinical Trials and their Outcome

Compound	MMPI type	Company	Indication	Outcome
 Batimastat (BB-94)	Broad-spectrum peptidic hydroxamate	British Biotech Pharmaceuticals	Malignant ascites	Discontinued due to poor bioavailability [56]
 Marimastat (BB-2516)	Broad-spectrum peptidic hydroxamate	British Biotech Pharmaceuticals/ Schering-Plough/ Tanabe Sieyaku	Glioblastoma, breast cancer, ovarian cancer, small-cell and non-small cell lung cancer, and pancreatic cancer	Discontinued due to lack of efficacy [57, 58]
 Trocade (Ro 32-3555)	Selective collagenase hydroxamate	Roche Pharmaceuticals	Rheumatoid arthritis	Discontinued due to lack of efficacy [59]
 Tanomastat (BAY 12-9566)	Selective gelatinase non-peptidic biphenyl	Bayer	Small-cell lung cancer	Discontinued due to poorer results than placebo [60]
 Prinomastat (AG3340)	Selective gelatinase non-peptidic hydroxamate	Pfizer/Agouron Pharmaceuticals	Non-small cell lung cancer	Discontinued due to lack of efficacy [61]
 BMS-275291	Broad-spectrum non-hydroxamate	Celltech/Bristol-Myers Squibb	Non-small cell lung cancer Prostate cancer	Discontinued due to dose-limiting toxicities [62] Ongoing [63]
 Metastat (COL-3)	Chemically modified tetracycline	CollaGenex Pharmaceuticals	Kaposi's sarcoma	Ongoing

The biological function of MMPs has traditionally been thought to be the degradation and remodeling of the extracellular matrix proteins but recent evidence suggest that MMPs are also important in intracellular signaling as well as in the secretion, bioactivation and stability of cytokines and growth factors. In addition, by modifying ECM molecules, MMPs indirectly influence cell migration, proliferation, differentiation and apoptosis.

MMPs in Normal Physiology and Diseased States

MMPs are essential for extracellular matrix homeostasis in various tissues and participate in such diverse processes as reproduction, morphogenesis, embryonic development, bone remodeling, angiogenesis and tissue repair [2, 47]. This knowledge is largely based on the localization and expression, acquired by immunohistochemical and *in situ* hybridization studies, rather than the functionality of individual MMPs. Subsequently, studies on gene knock-out mice have supplemented this knowledge [48]. The phenotypes of different MMP-deficient mice strains are not as striking as would be expected probably because of the overlapping functions of MMP members [48]. One exception is the MMP-14 deficient mouse that shows severe skeletal abnormalities and dies early [49]. A transient reduced long bone growth secondary to impaired angiogenesis is observed in MMP-9 deficient mice [50]. Wound healing defects have been reported to occur in the skin of MMP-3 and in the mucosa of MMP-7 null mice [51, 52]. MMP-2 knock-out mice develop normally but show slower growth [53]. In fact, when challenged, the MMP-2 deficient mouse is also incapable of supporting normal new vessel growth [53, 54]. These findings suggest that gelatinases are involved in angiogenesis and that it may therefore be appropriate to target them selectively by synthetic MMP inhibitors to reduce tumor progression. Martignetti *et al.* [55] recently reported for the first time a MMP mutation in humans. Mutation of the MMP-2 gene of a Saudi-Arabian family resulted in osteolysis, intraphalangal erosions, joint contractures, nodular fibrous palmar and plantar pads, and dysmorphic facies. In addition, fibroblasts cultured from the skin did not produce MMP-2 and MMP-2 was undetectable in sera of these MMP-2 deficient patients [55].

In contrast, excessive MMP activity may be pathogenic in malignant processes, and in diseases characterized by inflammation such as arthritis, osteoarthritis, periodontitis and ulceration.

MMP Inhibitors (MMPI) and Treatment

Enormous efforts have been invested to therapeutically control destructive and pathogenic MMP activities. The patent literature encompasses several thousands of entities in this field today. The three major treatment modalities that have emerged are natural antibiotics, tetracycline derivatives without antibiotic activity and small-molecule synthetic MMPI. Synthetic inhibitors target zinc at the active site by introducing thiol, carboxyl, phosphorous and hydroxamate groups of non-peptidic or peptidic compounds [4]. Development focuses on increasing the stability and specificity of the MMPI [4].

The first generation MMPI were designed to target MMPs broadly and were tested predominantly in oncology. Preclinical tests with MMPI in animal models, commonly using human transformed cell lines implanted in mice, showed impressive efficacy of MMPI. Increased survival and even tumor regression were often observed after MMPI therapy. Based on these encouraging experimental results, several large-scale randomized clinical trials, involving about 200 patients in each treatment arm, were initiated in the mid 90's with different MMPI (Table 2). The results have been overwhelmingly disappointing so far and all trials reported to date have failed perhaps with one exception. The oral hydroxamate marimastat (BB-2516) increased survival (P = 0.07) in patients with un-resectable gastric adenocarci-noma. A subset of patients, that received chemotherapy prior to MMPI treatment, benefited the most [64]. The result reflects the current opinion that the animal tumor models used for MMPI screening were in a less advanced stage than those in man [65]. For example, batimastat (BB-94) showed no efficacy on advanced tumors in mice [66].

Another caveat is that before embarking on large-scale clinical trials the proof-of principle, i.e. inhibition of MMP activity at the local site of the disease process, needs to be verified [65, 67]. This is a difficult task because there are no reliable methods of analysis [65] and normally only a small proportion of the MMP is enzymatically active. Surrogate markers, such as collagen metabolites and growth factors, correlate poorly to efficacy [65]. Data are also accumulating suggesting that MMP levels increase with MMPI treatment possibly by preventing their degradation [68-70]. Another explanation to the many failures with MMPI therapy may be that the dosages (e.g. maximal tolerable doses 1200 mg) were adapted to levels that did not cause side effects, most commonly joint stiffness and swelling [58, 65, 71], and thus were not therapeutically effective. To circumvent this more specific MMPI have been developed which allow higher dosages. BAY 12-9566 is one example, the trial of which, however, was prematurely terminated because of poorer survival in active compared with placebo-treated patients with small-cell lung cancer [60]. It should also be emphasized that the proMMPs are often positioned on their substrates resulting in extremely high virtual substrate concentrations, conditions very different from those of the test tube experiments used for designing MMPI. It may be speculated that MMPI with fast reaction rates would be the most appropriate for this condition.

The only approved MMPI for human use is the oral doxycycline (Periostat®, CollaGenex Pharmaceuticals), in the treatment of adult periodontitis. Other indications for MMPI are desperately sought such as restenosis, cerebral hemorrhage, multiple sclerosis and inflammatory respiratory diseases [59, 72]. We have novel experimental data that favors exploration of yet another medical area, namely anastomosis repair after colorectal surgery, where MMPI might be beneficial [73].

COLORECTAL CARCINOMA, SURGERY AND COMPLICATIONS

Epidemiology

Colorectal cancer is the fourth commonest cancer worldwide [74]. Estimates of the worldwide incidence 1990

were 783,000 patients with colorectal cancer distributed about equally between men and women [74, 75]. Rudy *et al.* [75] reported an estimate of 129,400 new colorectal cases in the US 1999. In Denmark 2002, the incidence of colorectal cancer was 3,500 per 5 million, and a trend towards an increasing occurrence of colorectal cancer has been reported in Western Europe [76]. There are regional differences and in Africa colorectal cancer is less common than in the rest of the world.

Treatment

Radical surgical removal of the tumor constitutes the crucial part of treatment. There is an increasing trend towards supplementary treatment with chemotherapeutics in patients with an unfavorable prognosis, or radiotherapy in lower rectal cancer. Various surgical techniques may be adopted for the completion of the intestinal anastomosis following colonic or rectal resection. The classical technique involves hand sewing of the anastomosis, whereas automatic stapling devices are more recent developments allowing faster and easier anastomotic construction.

Surgical Complications

One of the most feared complications following gastrointestinal surgery is anastomotic dehiscence that is associated with high morbidity and mortality. In elective surgery, clinically proven leakage is reported to occur in up to 11% of colonic anastomosis [77]. The incidence is lower after resection of the right side of colon compared with left side [78]. Anastomosis after rectal resection or emergency procedures on the colon mainly due to acute obstruction or peritonitis after intestinal perforation is particularly associated with a high risk of clinical leakage [79]. The Danish Colorectal Cancer Group recently reported an incidence of anastomotic dehiscence of 13% in a cohort study from 1994 to 1999 encompassing in total 5,021 patients with first-time rectal adenocarcinomas [80]. Furthermore, anastomotic leakage occurred more frequently in males than in females [80].

The causes of these severe complications are usually multifactorial. Retrospective studies have identified malnutrition, hypertension, diabetes, advanced age, male gender, leukocytosis, alcohol abuse, smoking, immunosuppression, irradiation, intraoperative septic conditions, duration of operation, and transfusion due to blood loss as risk factors of developing anastomotic leakage [81-86]. One measure to prevent these complications may include emptying the bowel content before surgery. However, an updated systematic review failed to demonstrate the benefit of mechanical bowel preparation [87]. Tension of the intestinal tissue and perfusion at the intestinal resection margins also determine the surgical result although no advantage was found with stapled over handsewn colorectal anastomosis with respect to anastomotic leakage [88]. A temporary fecal diversion is often carried out through the creation of an ileostomy or a colostomy to reduce the risk of anastomotic dehiscence. This surgical procedure necessitates a secondary operation to reestablish the intestinal continuity.

We are unaware of any documented pharmaceutical intervention designated for anastomotic dehiscence prevention.

EXPERIMENTAL COLON ANASTOMOSIS REPAIR

Healing Mechanisms and Collagen Metabolism in Colon Anastomosis

Because of the nature of this surgical procedure the molecular and biochemical processes responsible for anastomosis dehiscence have been studied almost exclusively in experimental models. Exhaustive reviews on the basic anastomotic repair mechanisms have been published by Hendriks and Mastboom [89] and by Koruda and Rolandelli [90].

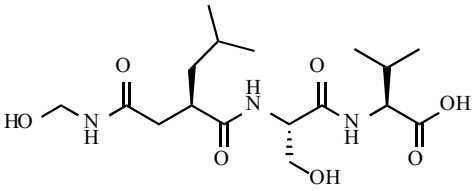
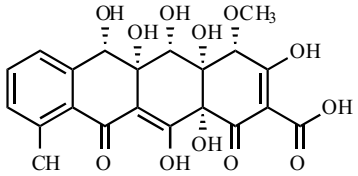
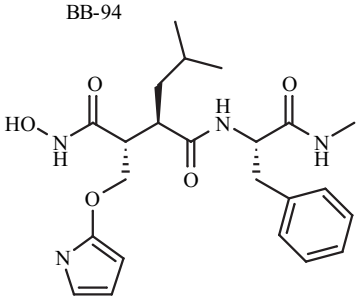
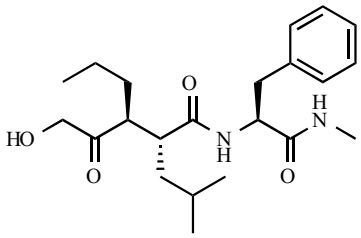
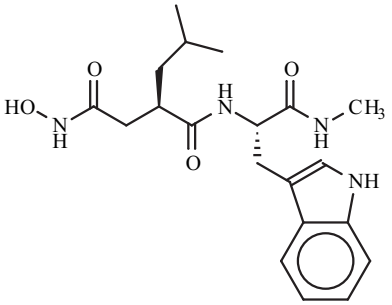
The diagonally arranged collagen network in the submucosal layer of the colon imparts tensile strength and retains the sutures of an anastomosis [91-93]. The anastomotic biomechanical strength declines in the early postoperative course in rats and in adult dogs [94-97]. A minimum in anastomotic strength is reported consistently to occur on postoperative day 3 [89, 94-97]. Interestingly, in patients, the initial symptoms of anastomotic leakage are usually detected around postoperative days 3-4. The reduced tensile strength is paralleled by a dramatic loss of existing collagen molecules in the anastomotic wound [94-96, 98, 99]. After day 3, the anastomotic strength and collagen synthesis increase rapidly [94-96, 100]. Collectively, in the early postoperative phase the breakdown of existing collagens in the tissue that retains the sutures exceeds the synthesis of new immature collagen molecules that results in weakened anastomotic strength. This strongly suggests the involvement of collagen degrading enzymes in the pathogenesis of anastomosis dehiscence.

MMPs in Colon Anastomosis Repair

Generally the expression and activity of MMPs increase after tissue injury although the temporal and spatial pattern varies among the different MMPs [101-105]. Inflammatory cells appear to be the predominant source of MMPs (MMP-8 and MMP-9) in the early inflammatory phase of tissue repair [46, 106]. Increased MMP levels and activities have also been demonstrated in anastomotic wounds by a number of techniques such as immunohistochemistry [107-109], *in situ* hybridization [110], functional activity assays [111] and zymography [109, 112]. MMPs are further elevated in the presence of local infection [113] and colon obstruction [108, 114].

We have carried out detailed biochemical and immunohistochemical studies on the localization of overall endogenous MMP activity and specific MMPs in the anastomotic wound on day 3 in the left colon of male rats. Colon tissue biopsies were dissected out from the areas around the suture channels and from adjacent non-suture holding wound area at –20°C. Biopsies were then incubated *in vitro* in assay buffer at 37°C for 24 hours [5]. Collagen degradation was expressed as the quotient between hydroxyproline, indicator of collagen, in media and in remaining non-fragmented tissue [114].

Table 3. Preclinical Results of MMP Inhibition on Colonic Anastomotic Biomechanical Strength in Male Rats Arranged in Escalating MMPI Dosages

Study	MMPI	Dosage and route	Day of assessment	Efficacy*
Kiyama *et al.* [122]	BE16627B 	8 mg/kg s.c.	Day 4	28% ↑ bursting pressure
Siemonsma *et al.* [123]	Doxycycline 	15 mg/kg bid s.c.	Day 3	26% ↑ breaking strength 98% ↑ bursting pressure
De Hingh *et al.* [97]	BB-94 	30 mg/kg i.p.	Day 3	27% ↑ breaking strength 54% ↑ bursting pressure
Syk *et al.* [96]	BB-1101 	30 mg/kg s.c.	Day 3	48% ↑ breaking strength
Ågren *et al.* [73]	GM6001 	100 mg/kg s.c.	Day 3	99% ↑ breaking strength

*↑Denotes significant ($P < 0.05$) increase in biomechanical strength parameter (breaking strength/bursting pressure) compared to vehicle-treated control rats.
¶ Bacterial-derived hydroxamate MMPI.
s.c. = subcutaneous administration. i.p. = intraperitoneal administration.

Degradation of colon collagens by active MMPs in the anastomosis was substantially elevated, at least 10-fold, in the suture-holding area of the anastomotic wound compared to adjacent non-sutured area. The metal chelators ethylenediaminetetraacetic acid and 1,10-phenanthroline blocked this activity completely. Negligible endogenous collagenolysis was detected in proximal uninjured colon. Elevated MMP activity was reflected in a 30% drop in tissue hydroxyproline concentration in sutured area compared with adjacent non-sutured area. This finding underscores further the pathogenic role of one or more MMPs on the autodestruction of suture-holding collagen molecules leading to increased risk of anastomotic dehiscence.

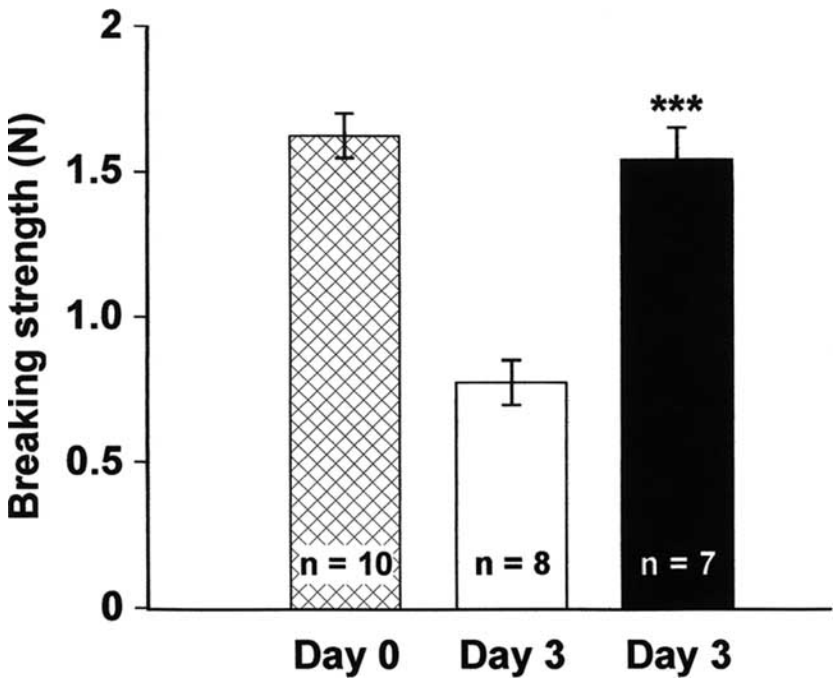


Fig. (1). Effect of the broad-spectrum synthetic MMP inhibitor (MMPI) GM6001 (solid bars) on breaking strength of left-sided colon anastomosis 3 days after it was constructed compared to suture-holding capacity on day 0 (crosshatched bars) and to vehicle-treated control rats (open bars) day 3. n denotes number of animals and anastomoses. The body weight of the male rats was 246 ± 4 g. *** $P <$ 0.001 compared with vehicle day 3 (*t*-test). Mean ± SEM.

As mentioned above, neutrophils are rich sources of MMPs in the acute phase of tissue injury and neutropenia appears beneficial for early anastomosis integrity [115]. A massive neutrophil infiltration is also observed in the colonic anastomotic wound day 3. The neutrophil-derived gelatinase B or MMP-9 is also up-regulated in colon anastomotic wounds [107, 112]. By the use of immunohistochemical analysis, we could confirm the presence of MMP-9 and further demonstrate that the neutrophil collagenase-2 or MMP-8 was present in the extracellular compartment of the wound but not in uninjured rat colon day 3. Previous *in vitro* studies indicate that collagenases and gelatinases act in a synergistic way in collagenolysis of cartilage [116]. To test the hypothesis that MMP-8 and MMP-9 degrade colon collagens in concert, recombinant and aminophenylmercuric acetate-activated MMP-8 and MMP-9 were added individually or together to biopsies of normal rat colon that were incubated *in vitro* [116]. Although both MMP-8 and MMP-9 displayed some collagenolytic activity the synergistic effect of MMP-8 and MMP-9 was striking and collagenolysis was augmented three-fold compared to the contribution of MMP-8 and MMP-9 alone. These findings suggest that collagenolysis in the anastomotic wound is achieved by at least two types of MMPs, the collagenases that make the initial site-specific cleavage of intact collagen molecules followed by further degradation of the denatured collagens by the gelatinases.

MMP Inhibition in Experimental Colon Anastomosis Repair

A large body of evidence then suggests that the decreased anastomotic strength is MMP-mediated. We therefore next carried out series of animal studies using the broad-spectrum hydroxamate MMPI, GM6001 [117] to inactivate the MMP activity and improve biomechanical properties of the anastomosis. The molecular structure of GM6001 is shown in Table 3.

GM6001 (100 mg/kg) or vehicle was injected daily and subcutaneously (s.c.) in male Sprague-Dawley rats (220-360 g) starting 2 days before operation [73]. Left-sided colon anastomoses were made as described by Syk *et al.* [96]. Breaking strength was determined immediately after the anastomosis was made in non-treated operated rats or on postoperative day 3 in treated rats [95]. GM6001 concentrations in whole blood and in resected anastomotic wound segment were about 1.5 μM 24 hours after the last GM6001 injection. The breaking strength was significantly higher ($P < 0.001$) in GM6001-treated compared to vehicle-treated rats on postoperative day 3 in two separate series [73]. Furthermore, the anastomotic breaking strength in GM6001-treated animals did not differ significantly ($P =$ 0.55) from the strength of the anastomosis immediately after surgery (suture-holding capacity) while it was lowered by 50% in vehicle-treated controls (Fig. (**1**)). The latter finding strongly implies that GM6001 completely blocked fragmentation of the existing colon collagen molecules in the anastomosis. No side effects were observed with the GM6001 treatment. In another study using the same GM6001 dosing schedule, postoperative intraabdominal adhesions did not increase with this short-term MMPI treatment in a standardized experimental model [118, 119]. Witte *et al.* [120] observed increased skin tensile strength despite a slightly slower body weight gain after 11 daily GM6001 injections at 100 mg/kg compoared to vehicle-treated rats. Balcom *et al.* [121] administered batimastat (BB-94) at 40 mg/kg intraperitoneally for 14 consecutive

days without observing any untoward effects on either healing of primary skin wounds or of small intestinal anastomoses.

Four other experimental studies investigating the efficacy of MMP inhibition on anastomotic healing have been reported in the literature (Table 3). In two of them hydroxamate-based broad-spectrum MMPI were used [96, 97], in one a bacterial-derived hydroxamate MMPI [122] and in another doxycycline [123]. From Table 3 it is clear that MMPI treatment significantly improves the biomechanical properties of colon anastomosis under uncomplicated conditions. Our results appear superior to those reported in the other four studies. One explanation could be that we used a higher dose of MMPI. In support of this, systemic GM6001 treatment effectively inhibited MMP activity by more than 90%, as measured by the collagen degradation marker ICTP (carboxyterminal telopeptide of type I collagen) in serum [124], while doxycycline at 40 mg/kg a day only marginally depressed ICTP serum levels [125].

To explore MMPI therapy in a more clinically related situation we are at the moment testing synthetic MMPI in an experimental model of acute colon obstruction [114, 126]. These findings, if reproduced in surgical patients, could open up for new surgical strategies in that primary anastomosis may become safer and eliminate the need for construction of a temporary or permanent intestinal stomy.

SUMMARY

Our *in vitro* and *in vivo* data strongly suggest that dehiscence of colon anastomosis is MMP-dependent. Further experimental and pharmocodynamic work is warranted to identify the optimal MMPI and dose regimen to achieve maximal healing of intestinal anastomoses. Prevention of colorectal surgical complications is a promising indication for MMPI therapy. The future perspective is to explore the therapeutic value of MMPI in colorectal patients and active search of an industrial partner is ongoing.

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ABBREVIATIONS

MMPs, matrix metalloproteinases; MMPI, matrix metalloproteinase inhibitor(s); ECM, extracellular matrix; TIMPs, tissue inhibitors of metalloproteinases; ICTP, carboxyterminal telopeptide of type I collagen.

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