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Matrix metalloproteinase inhibitors: Present achievements and future prospects

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Abstract

Matrix metalloproteinases (MMPs) are a class of structurally related enzymes that function in the degradation of extracellular matrix proteins that constitute the pericellular connective tissue and play an important role in both normal and pathological tissue remodelling. Increased MMP activity is detected in a wide range of cancers and seems correlated to their invasive and metastatic potential. MMPs thus seem an attractive target for both diagnostic and therapeutic purposes.

Several synthetic matrix metalloproteinase inhibitors (MMPIs) are currently being developed. Preclinical studies are promising as they suggest inhibition of several steps in the metastatic process. Marimastat is the first MMPI to enter comparative phase III trials after early clinical trials established the safety profile. Clinical trials will need to be specifically designed to optimally evaluate the therapeutic potential of this novel class of cytostatic drugs. Safety studies should consider the markedly different toxicity profile and determine the range of biologically active dosage, while efficacy studies should be performed in selected clinical settings with appropriate end-points. We review the present achievements in preclinical and clinical studies with MMPIs, discuss specific considerations for appropriate study design and reflect on the future prospects of this novel class of agents.

Introduction

Matrix metalloproteinases (MMPs) function in the degradation of extracellular matrix proteins that constitute connective tissue. They are one of the four major classes of proteases that play a role in the evolution of cancer. Proteases in general play a role in many diseases. The activity of most extracellular proteases is controlled by specific naturally occurring inhibitors. The importance of these inhibitors can be illustrated by the identification that their deficiency is related to specific disease or disorders. Deficiency of for instance antithrombine III relates to thrombosis, and of a-antitrypsine to emphysema.

Proteases

Extracellular proteases are essential for tumor cells to be able to penetrate the basement membrane. Proteolytic degradation of the extracellular matrix (ECM) is also necessary when invasive tumor cells penetrate tissues, gain acces to blood vessels, exit blood vessels and colonise distant sites (metastasis). In addition, angiogenesis (a neovascularization process crucial to sustain tumor growth) involves activity proteolytic degradation of the ECM by invasive endothelial cells [1]. The four classes of endopeptidases that play a role in these processes are serine, cysteine, aspartyl and metalloproteinases [2]. Of the serine peptidases, urokinase type plasminogen activator (uPA) and tissue type plasminogin activator (tPA) known to be produced by many tumor cells [3, 4], generate plasmin from plasminogen. Plasmin, in turn, is a know activator of several MMPs. These are a family of structurally related enzymes that

Table 1. Common features of MMPs

1.	Produced in an inactive form
2.	Two Zn^{2+} atoms, including one at the active site
3.	Two CA^{2+} ions, essential for enzyme-stability
4.	Primary structure typically contains two highly conserved regions, 1 each in the N-terminal propetide domain and in the catalytic domain
5.	Inhibited by specific inhibitors known as tissue inhibitors of metalloproteinases (TIMP)

contain a zinc atom at their active site (Table 1). As early as in 1962, interstitial collagenases, responsible for the resorption of tadpole tails [5], was the first MMP to be described and purified. MMPs are secreted as latent pro-enzymes and are activated by proteolytic removal of an amino-terminal domain [6]. MMPs can be classified on the basis of sequence homology and substrate specificity into four groups (Table 1). The first group consists of three collagenases: interstitial collagenase, neutrophil (PMN) collagenase, and collagenase-3 which specifically degrade type I, II, and III fibrillar collagens [7–9]. The second group consists of the 72 kDa and 92 kDa gelatinases, also referred to as type IV collagenases [10, 11], and metalloelastase. The substrate specificity of the gelatinases is broader than that of the collagenases. Both gelatinases will degrade the type IV and V non-fibrillar collagens of basal lamina, and have also been reported to degrade type VII collagen, fibronectin [10], type X short chain collagen [12] and elastin [13]. The latter is the substrate for metalloelastase. The third group of MMPs consists of two closely related enzymes, stromelysin-1 and stromelysin-2 [14, 15] and a more distantly related enzyme, matrilysin (PUMP) [16]. They degrade a wide variety of proteoglycans, fibronectin, laminin, as well as type IV collagen [14] and type X collagen [17]. Stromelysin-3 [18] despite its name, does not neatly fit into this group [19]. Although mRNA *in situ* hybridisation studies suggest that stromelysin-3 is important in malignant disease the proteolytic function of the native protein has yet to be described. Recent studies with purified recombinant mouse stromelysin-3 have revealed weak stromelysin/proteoglycans activity.

With the exception of fibrillar collagenases, the preferences of these enzymes for particular matrix substrates *in vivo* is poorly defined. However, it is clear that, collectively, matrix metalloproteinases are capable of degrading all of the components of the extracellular matrix.

Recently, membrane-type metalloproteinases (MT-MMP) were discovered [20]. MT-MMP is a membrane-bound enzyme with recognized transmembrane domain. It appears to specifically activate progelatinase A [21]. Since the initial discovery, genes for three other MT-MMPs have been identified and these enzymes now constitute the fourth group of MMPs [22]. MT-MMP is the only MMP so far identified that is not a secreted product.

Many published reports have documented the preferential localisation of proteases in adjuvant stromal cells, rather than in invasive malignant cells. This suggests that tumor cells can trigger the production of proteases by the surrounding stromal cells [18, 23–25]. This concept is supported by the partial characterisation of a tumor cell-derived factor that stimulates normal cells to produce collagenase, stromelysin and gelatinase A [26].

Invasive cancer cells thus have the ability to increase their proteolytic activity without increasing their own production and secretion of proteases, and they can concentrate and activate proteases in the pericellular space. By producing a variety of proteases they can also achieve optimal matrix degradation. This provides them with routes of escape against attempts to control the production and secretion of proteases in cancer cells. Because of this, inhibition of membrane-bound activated proteases or of binding pro-enzyme to membrane associated receptors may represent important alternative ways to inhibit tumor cell proteolysis [27, 28]. It may be important to target more than one family of proteinases.

Matrix metalloproteinases in cancer

Normally, the degradative activity of MMPs is tightly controlled both by the latency of the secreted enzymes as well as by the presence of naturally occurring inhibitors. The latter include general plasma proteinase inhibitors such as a_2-macroglobulin, as well as more specific inhibitors such as the tissue inhibitors of metalloproteinases, TIMP-1 [29], TIMP-2 [30] and TIMP-3 [31]. However, in several diseases such as arthritis [32], neurodegenerative disease such as multiple sclerosis [33] and cancer [34], there appears to be a local and temporal imbalance between the levels of activated enzymes and their inhibitors. This imbalance results in a breakdown of the extracellular matrix. Several studies have shown that in cancer models the proportion of active MMPs overwhelms the local inhibitory activity

Table 2. Matrix metalloproteinase family

Matrix metalloproteinase	MMP number	Preferred substrate
Class I		
Interstitial collagenase	1	Fibrillar collagens, type I, II, III
Neutrophil (PMN) collagenase	8	Fibrillar collagens, type I, II, III
Collagenase-3	13	Fibrillar collagens, type I, II, III
Class II		
Gelatinase A (72 kDa)	2	Collagen types IV, V, gelatin
Gelatinase B (92 kDa)	9	Collagen types IV, V, gelatin
Metalloelastase	12	Elastin
Class III		
Stromelysin-1	3	Laminin, fibronectin, proteoglycans
Stromelysin-2	10	Laminin, fibronectin, proteoglycans
Matrilysin (pump)	7	Laminin, fibronectin, proteoglycans
Non-Classified		
Stromelysin-3	11	1-antritrypsin?
Membrane-type MMP	14	Pro-gelatinase A

surrounding the tumor. This MMP activity facilitates the direct expansion and local invasion of the primary tumor, the movement of tumor cells across the vascular basement membrane, and the local growth and invasion of any secondary tumors. MMP activity also contributes to the invasive ingrowth of new blood vessels, a requisite for malignant tumor growth.

It is unlikely that every type of cancer uses MMPs to break down matrix barriers, and other classes of proteases, such as the urokinase-type plasminogen activator, are known to be expressed in high levels in certain malignancies. One of the major challenges in the development of matrix metalloproteinase inhibitors (MMPIs) as anticancer agents is to identify which cancers utilize MMPs and therefore might respond to MMPI therapy. It is of utmost importance to realize that very few human cancer cell lines and experimental *in vivo* models reflect the MMP expression of the tumor from which they were isolated. Therefore human tissues taken at the time of primary surgery provide the best insight into the role of MMPs in human cancer.

Collagenases

Fibrillar collagenase activity in epidermoid carcinomas of the oral cavity and larynx was found increased compared to the activity in normal mucosa [35]. High levels of fibrillar collagenase activity were also found at the invading edge of gastric carcinomas [36], and in colorectal cancer a correlation was detected between collagenolytic activity and grade of histological differentiation [37]. Further, interstitial collagenase in the connective tissue stroma of colorectal carcinomas was increased as compared with the staining in adenomas and normal mucosa [38]. The cellular source of the collagenase in these tumor tissues has long been a subject of debate. Recent studies have localized mRNA encoding interstitial collagenase to both invasive carcinoma cells and stromal fibroblasts in epidermoid cancers of the head and neck [39], and in 9 out of 22 lung cancers. mRNA encoding interstitial collagenase were not detected in nine cases of prostate cancer [40].

Stromelysins

A high level of mRNA encoding for stromelysin-1 has been detected in lung- and in head and neck cancer [41], both in stromal and tumor cells. It was not found in prostate [40], gastric or colorectal cancer [42]. In contrast, high levels of PUMP-1 mRNA expression were revealed in gastric and colorectal cancers [42]. In the latter it was localized in tumor tissue or in adjacent stroma, with little or no expression in surrounding normal tissue [18, 43]. High levels were also found in prostate cancers, localized to the malignant and nonmalignant epithelial cells but not found to the associated stroma [40].

Gelatinases

High levels of activated gelatinase A were demonstrated in invasive cancer of the breast [44], while the ratio of activated to latent gelatinase A was significantly higher in malignant versus benign breast lesions and a higher proportion of activated enzyme was related to increasing tumor grade [45]. The expression of gelatinase A was correlated with the progression of colorectal, gastric, and breast cancer [46], but failed to predict relapse or survival in patients with node-negative breast cancers [47]. Gelatinase A was also found to be higher in invasive ovarian cancer than in benign cystadenomas of the ovary, and was particularly intense around micro-invasive cells or clusters [48]. It was significantly elevated in sera of lung cancer patients as compared to normal sera, and levels were also significantly higher in patients with distant metastases versus those without distant metastases [49]. In non-small cell lung cancer and matched non-involved lung tissue there was also a highly significant correlation between the level of expression of gelatinase A and histopathological evidence of tumor spread [50].

Gelatinase A was localized to the cells of the tumor-associated stroma in both colorectal cancer [23] and infiltrating squamous cell cancer of the skin [51]. In the latter study, expression of the mRNA encoding gelatinase B was localized to the squamous cancer cells at the interface between tumor and stroma and was also detected in a subpopulation of tissue macrophages.

Stromelysin-3

There have been several correlative studies in breast cancer showing high levels of expression of stromelysin-3 mRNA in tumor tissue or in adjacent stroma, with little or no expression in surrounding normal tissue [18].

Only low levels of stromelysin-3 mRNA were detected in colon, ovary, kidney and lung cancers. More recently stromelysin-3 mRNA and protein have been detected exclusively in the stromal cells surrounding squamous cell cancers of the head and neck, and these levels were significantly correlated to the invasiveness of the cancer cells. In the samples, transcripts for stromelysin-3 were localized exclusively in the neoplastic cells, while those for interstitial collagenase were found in both stromal and neoplastic cells [3]. These results point to an important role for stromelysin-3 in tumor progression and make it even more important to understand the proteolytic function of this metalloenzyme.

MT-MMP

MT-MMP, the membrane-bound enzyme with a recognized transmembrane domain, appears to be a specific activator of progelatinase A [21]. The expression of MT-MMP by cancer cells would confer the ability to activate gelatinase A produced locally by stromal cells at the invasive tumor margins. In a study of gastric cancer, MT-MMP was expressed exclusively in the tumor tissue and co-localised with activated gelatinase A in invasive carcinoma cell nests [52].

Matrix metalloproteinase inhibition

Both native and synthetic inhibitors have been considered for therapeutic aims. The native inhibitors TIMP-1 and TIMP-2, are potent broad spectrum inhibitors (inhibiting collagenases, stromelysins and gelatinases) and have been in development as therapeutic agents since their identification and sequencing in 1985 (TIMP-1) [29] and 1989 (TIMP-2) [30]. However, the therapeutic use of these proteins is likely to be limited by their low oral bioavailability. Their size, 28 kDa and 21 kDa respectively, may also limit tissue penetration. Presently efforts are underway to characterize the functional domains of the TIMP molecules [53, 54], hopefully yielding new synthetic or recombinant approaches.

Synthetic matrix metalloproteinase inhibitors (MMPIs) have been developed since the early 1980s. The majority of these inhibitors are substituted peptide derivatives, analogues of the cleavage site in the collagen molecule with a metal-binding group in the position of the cleaved peptide bond. the metal binding group, commonly a thiol, sulphydryl, carboxyl or hydroxamate group, binds to the zinc atom in the activity site of the matrix metalloproteinase [55]. Potencies have been reported in the low nanomolar range and it has recently proved possible to design selective inhibitors as well as broad spectrum agents. Selective inhibitors are likely to be pursued with greater intensity should long term toxicology limit the use of broad spectrum agents.

Preclinical studies

It is of importance that *in vitro* studies with MMPIs demonstrated absence of direct cytotoxicity. MMPIs were first considered as potential antimetastatic agents. This antimetastatic activity had been demonstrated in several experimental *in vivo* models of organ colonisation by intravenously inoculated tumor cells. Intraperitoneal administration of recombinant TIMP-1 and TIMP-2 inhibited the colonisation of lungs by both B16-F10 murine melanoma cells [56] and ras-transfected rat embryo 4R cells [57]. Similar results were obtained with the synthetic hydroxamate MMPI SC44463 which yielded a > 90% inhibition of lung colonisation by B16-F10 cells [58]. In addition, intraperitoneal administration of the synthetic hydroxamate MMPI BB-94 (batimastat) was reported to significantly inhibit lung colonisation and tumor growth of B16-BL6 murine melanoma cells [59] and inhibit by up to 80% both the number and median weights of lung colonies by HOSP.1P rat mammary carcinoma cells [60]. This implied an effect on both seeding efficiency and subsequent tumor growth.

However, these models only reflect the final stages of hematogenous metastatic spread and do not provide information on the effects of these inhibitors on lymphatic spread. In a spontaneous metastatic model, in which batimastat was given when subcutaneous tumors were established until surgical removal or at time of removal at day 70, lymphatic dissemination was less susceptible to inhibition than hematogenous spread. However, long-term treatment could prevent the outgrowth of both lymphatic and lung micrometastasis, allowing sustained tumor-free survival. These results suggest, that MMPI treatment may directly suppress growth and development of lymphatic metastasis, while the anti-angiogenic properties of these drugs may relate to the inhibition of hematogenous metastates [61].

Further studies have now provided a basis for the development of MMPIs as both antitumor growth and anti-angiogenic agents. In experiments with TIMP-2 cDNA transfected rat embryo 4R cells it was shown that production of TIMP-2 markedly reduced tumor growth following subcutaneous implantation [62]. Treatment with batimastat in a human ovarian carcinoma xenograft ascites model in mice resulted in important reduction of tumor burden, reduced tumor growth and increased survival [63]. Transition of the thick muscinous ascites to a solid avascular tumor was associated with encapsulation of the clumps of tumor cells by host stromal cells. This was proposed to be caused by inhibition of intraperitoneal MMP activity with shifting of the balance from ECM breakdown to formation of stromal connective tissue. The weakly active diasteromer of BB-94 showed no antitumor activity [63]. There was no evidence of vascularization and there were signs of tumor cell necrosis.

Batimastat was further evaluated in several other xenograft models. Inhibition of organ invasion was reported in two human colorectal xenograft models [64]. In a hepatic invasive model (C170HM$_2$-cell line) batimastat significantly reduced the number and size of liver metastasis, while in the lung invasive model (AP5LV-cell line) a significant reduction of tumor weight within the lung, but not in the number of nodules was detected. In an orthotopic transplant model, human colon-carcinoma xenografts were orthotopically implanted on the colon in mice. Intraperitoneal treatment with batismastat not only reduced the number of livermetastasis, but also caused a significant reduction in the median weight of the primary tumor and incidence of local and regional invasion. This resulted in improved survival [65]. To evaluate an adjuvant therapeutic potential, batimastat was administered to mice after orthotopic human breast cancer xenografts (MDA-MD-45) were resected [9]. Significant inhibition of locoregional tumor regrowth and reduction of incidence, number and total volume of lung metastasis was demonstrated. No effect was seen on cancer cell viability *in vitro* and batimastat induced no major change in individual MMP (72kDa type IV human collagenase, stromelysin) or TIMP-2 gene expression, as judged by steady-state mRNA levels. This suggests that the inhibitory effect is solely due to MMP inhibition [66].

A study using a human colorectal cancer (C170HM$_2$-cell line) ascites model in mice also showed reduction of ascites formation and solid peritoneal tumor deposits [67]. However, another human carcinoma ascites model showed conflicting results. In this study, human breast cancer cells (MDA435/LCC6) were used to generate malignant ascites in nude mice. Treatment with batimastat resulted in major tumor cell consolidation with less dispersed ascites cells, but with evident residual tumor cell adhesions to peritoneal surfaces excluding significant reduction of overall tumor burden. No increase in survival or significant suppression of ascites formation was found [68].

Of importance for potential clinical studies is the observation that CT1746 (a gelatin selective synthetic MMPI) in combination wit the cytotoxic agent

cyclophosphamide was more effective than either agent alone in the treatment of murine Lewis lung carcinoma, both for the delay in growth of the primary lesion as well as for the number and size of lung metastates [69].

Collectively these observations support the hypothesis that MMPIs can act as antitumor agents by preventing remodelling of the extracellular matrix by tumor cells. This action also appears to underlie the mechanism by which MMPIs can exert an anti-angiogenic effect. *In vitro* studies have shown that both TIMP-1 and antisera to 72 kDa gelatinase can inhibit the invastion of extracellular matrix by capillary endothelial cells [1]. TIMP-1 has also been shown to inhibit tumor-induced vascularisation of the rabbit cornea [70], presumably by blocking the remodelling of the extracellular matrix that precedes new capillary growth. These more recent studies broaden the range of action of MMPIs.

We conclude that the reported preclinical studies indicate that MMPIs not only have an antimetastatic effect, but also inhibit steps in the whole metastatic cascade such as invasive tumor growth and angiogenesis. Whether tumor-MMP production is predictive for effect or site-specificity exist remains to be further elucidated.

Clinical studies

Presently, few MMPIs have yet been evaluated in clinical trials. Apart from specifically designed synthetic MMPIs, there has also been recent clinical interest in the use of tetracycline derivatives as antimetastatic agents on the basis of their weak collagenase inhibitory activity [71].

Batimastat was the first MMPI to be tested in cancer patients. Because of the demonstrated low bioavailability of batimastat oral administration was disregarded and other ways of delivery routes were tested. Deducted from the earlier described ovarian cancer xenograft model, batimastat was tested in patients with malignant effusions. A phase I study of intraperiotoneal administrated batimastat in patients with symptomatic malignant ascites was performed [72]. Patients with any form of malignancy who required paracentesis for symptomatic relief were eligible. A single intraperitoneal dose (150–1350 mg/m^2) of batimastat, suspended in 5% dextrose, was given following drainage. The treatment was generally well tolerated. Short lasting mild abdominal pain associated with nausea and vomiting was experienced by half of the patients. Treatment resulted in postponement of paracentesis in several patients. The intraperitoneal administration of a single dose of 1000 mg/m^2 resulted in high peak plasma levels (C_{max} 1000 ng/ml) and sustained plasma concentrations of 100–200 ng/ml after 4 weeks. The plasma concentration time curve (AUC) was linear with dose. The plasma half-life was 19 days. In a similar setting in patients without ascites, batimastat was given at doses of 600–1800 mg/m^2, once every 4 weeks [73]. Maximal plasma levels were reached within 24–48 hours following dosing, and levels > 160 ng/ml were maintained for 28 days. Systemic toxicities were minimal to mild and similar to the ones reported above. Local toxicities were of concern and included peritoneal irritation, abdominal pain requiring narcotic analgesia, vaso-vagal reactions and 1 case of bowel obstruction. No significant changes were seen in the peripheral blood activity of MMP-2 and -9. Four of nine patients had stable disease lasting from 3–8 months. Although a phase II study on i.p. batimastat was performed and suggested some benefits in patients with ovarian cancer, further development of batimastat was halted for this indication, in view of the local side effects of administering the drug this way.

A similar approach was also taken in patients with a symptomatic malignant pleural effusion [74]. Intrapleural batimastat was given at lower doses (15–300 mg/m^2) but nevertheless also resulted in a reduced frequency of pleural aspirations. Side-effects were mild and mainly consisted of some local discomfort. Peak plasma levels achieved ranged from 12–170 times the IC_50 of MMP-2 and -9, 6–115 times the IC_{50} of MMP-9 and 2–20 times that of MMP-3.

Objective tumor regression was not reported in either study, which is in part (obviously) the resultant of the chosen study design. These modes of administration are also limited by being impractical for a widespread use. Since MMPIs in view of their mechanism of action require continuous exposure for efficacy, present clinical development of MMPIs is again focused on oral administration. This also relates to an increased knowledge on the crystallographic structure of MMP-inhibitor complexes enabling a more focused design of molecules with selectivity for specific MMPs, while it also allowed change in the physicochemical properties of the inhibitor intended to improve bioavailability through the oral route [75].

Marimastat was the second MMPI to be evaluated in cancer patients. Similar to batimastat and SC 44463 it binds to zinc in the MMP through hydrox-

amic acid. A phase I study in healthy volunteers with a single oral dose of 200 mg marimastat showed very high peak plasma levels (C_{max} > 750 ng/ml) and indicated a terminal half life of approximately 9.5 hours. Using a twice daily (bid) oral schedule of 50, 100 and 200 mg for 6 days, the drug was well tolerated and 50 mg bid was sufficient to achieve higher than MMP-inhibitory IC_{50} levels [76]. Pharmacokinetic (PK) data in patients with metastatic lung cancer using an interpatient escalated (25–50 mg) bid oral schedule confirmed inhibitory C_{max} levels (180–580 ng/ml) with a terminal half life of approximately 5 hours. No detailed data on oral bioavailability nor PK data after prolonged administration were given [77].

The first dose finding phase I/II studies with marimastat, depicted as 'cancer antigen studies' involved patients with advanced ovarian, colorectal, pancreatic or hormone-refractory prostate cancer that were treated in similarly designed studies [78–84]. Inclusion criteria included progressive disease defined as a tumor-marker rise of $\geq$ 25% over the 4-week period prior to study entry. Patients received an inter-patients escalated (5 mg o.d. to 75 mg bid) oral schedule of marimastat for 4 weeks or until dose-limiting toxicity. Patients who failed to demonstrate a continuing rise (> 25%) of tumormarker levels were considered for long-term treatment continuation.

Preliminary results were interesting. The respective tumor markers CA 125, CEA, CA19.9 and PSA, were all found to behave quite similarly under marimastat therapy. In more than 50% of the patients treated for 4 weeks a diminished rise or an actual reduction of marker level was detected.

A "meta-analysis" on 360 patients entered into these studies showed marimastat to be maximally effective at a dose of 10 mg bid and higher [85]. At 10 mg bid the mean 12 hour marimastat plasma level of 81.9 ng/ml was 40 times higher than the IC_{50} of marimastat for collagenase and gelatinase [86]. The inhibitory effect on marker levels as described was statistically significant and radiographic and clinical assessment of patients on continued treatment indicated a possible stabilisation of disease, though without objective tumor regression. The drug was generally well tolerated and musculo-skeletal symptoms were the principal side effect noted after prolonged treatment. They consisted of pain and tenderness in muscles, tendons and joints, predominantly in shoulders and hands. The precise cause of these symptoms has yet to be established, but they may be related to an impairment of the normal process governing tissue remodelling. It occurred in about 30% of patients by five months of treatment at 10 mg bid and in 70% at 25 mg bid. The symptoms appeared to be dose-dependent, resolved quickly after drug withdrawal and did not preclude the possibility of continuation after a short drug holiday.

Additional analyses of the survival data of those patients continuing therapy beyond 4 weeks were made. There appeared to be an inverse relationship between the duration of survival and tumor marker progression at time of study entry. This was suggested by the authors as sufficient evidence to justify the use of marker progression as a prognostic feature of clinical outcome. In addition, a relationship was shown between the extent of reduction in marker-progression under marimastat treatment and the duration of survival with significant better survival outcome using a 10 mg bid schedule as compared to the suboptimal doses of 5, 10 or 25 mg once a day. It should be noted that these analyses were not defined prospectively, so the data suggesting a beneficial effect from marimastat on survival should be interpreted with caution.

Randomised comparative phase III trials of marimastat are planned in pancreatic cancer, small cell lung cancer, glioblastoma, gastric cancer and ovarian cancer. The study in pancratic cancer will compare different (5 mg, 10 mg and 25 mg) bid oral schedules of marimastat to standard gemcitabine as first-line therapy.

Another synthetic MMPI that has entered clinical phase I studies is CGS 27023 A, that shares the hydroxamic acid with marimastat as binding site to the zinc atom in the MMP. Data of the clinical studies have not been reported yet. Finally, reports on clinical studies tetracycline related drug doxycycline are soon to be expected.

In view of the major attention the clinical trials with marimastat have received in the lay-press, it appears worthwhile to discuss potentials, problems and practicalities of clinical trial design with MMPIs.

Clinical trial design

Clinical trials will need to be specifically designed to optimally determine the toxicity and the therapeutic potential of MMPIs, as they obtain their anti-tumor effects through a unique mechanism of action.

To design such "tailored" studies, we will need to find a balance between making optimal use of preclinical information on one hand and the realization of the

limited predictive value of this information for the clinical situation on the other. Early attempts to draw up specific guidelines for clinical trial design with MMPIs have already been made [87]. Undoubtedly, recommendations will be adjusted over time as more insight in biological effects and experience in clinical studies is gathered but it is useful to list the considerations to be made in a rational fashion.

Preclinical studies showed that MMPIs can rather be described as tumorostatic drugs as they inhibited invasive tumorgrowth, blocked neo-angiogenesis and reduced metastatic spread without showing any direct cytotoxicity. Translating these effects into a clinical setting requires a new concept in cancer therapy. Tumorostatic cancer therapy is an interesting alternative paradigm to be considered. The aim of such long-term therapy would be to create a state of tumor 'dormancy' by halting proliferation, and thus generating disease stabilisation.

Consequently, the main difficulties in designing clinical trials arise as no properly defined methods exist to evaluate such therapy.

Conventional cytotoxic drugs are designed to maximally reduce tumor burden and treatment effect can then be judged by well defined and standardised response criteria. No major tumor mass reduction is expected using MMPIs. Generally, early clinical trials are conducted to obtain a toxicity profile, recommend a therapeutic dose and establish clinical evidence of efficacy, preferably in a short time span.

The expected required long-term treatment with MMPIs requires minimal side-effects and an easy administration schedule in order not to limit drug compliance. The recommended therapeutic dose will thus not be based on the maximally tolerated dose, but on a dose with optimal biologic effect. We will have to anticipate, and trials with batimastat and marimastat have already confirmed this, that the toxicity profile of MMPIs will markedly differ from those of conventional cytotoxic drugs. Moreover, as mentioned several times, prolonged administration will be necessary. Because of this, phase I trials should be designed in such a way that subsequent cohorts of patients are not put at risk because of the unavailability of long-term results in the previous cohort. In addition it appears worthwhile to introduce some type of pharmacodynamic monitoring, since we are looking for the optimal biologic dose rather than the maximally tolerable dose. Present studies are all attempting to measure parameters such as bone turnover products, capillary blood flow (through doppler techniques) and angiogenesis (through PET scanning), and zymographic assays to determine plasma levels of MMPs. Obviously, in order to relate to pharmacodynamic endpoints, pharmacokinetic data obtained by making use of sensitive assays, will be crucial in all phase I and II studies. However, to detect if the high pericellular MMP activity is actually inhibited, direct tissue determinations may be more precise, yet are more difficult to acquire. Determining proenzymatic MMP serum levels may not be very informative as MMPIs inhibit MMP activity and not MMP secretion. These and other indirect parameters of MMP activity are under investigation.

As discussed, regression of bulky cancer is not to be expected by MMPIs. Such regression is commonly the focus of clinical phase II studies. It seems inappropriate to perform a wide variety of single agent phase II studies with MMPIs. As a single agent they might better be studied initially in phase III studies in patients with a very high likelihood of disease-recurrence, or disease progression after initially induced disease response by conventional cytotoxic agents. Development in classical adjuvant phase III studies would be too time-consuming.

In addition, and in view of some preclinical data, early combination studies of MMPIs with conventional cytotoxic drugs seem worthwhile pursuing. In this case, randomized phase II studies comparing the combination of the single agent cytotoxic drug would rapidly provide crucial information on the potential additive value of the MMPI. Tumor markers and other surrogate endpoints can helpful in guiding us through this process but will never be sufficient to provide convincing evidence of efficacy.

Conclusion

Matrix metalloproteinases appear to be important potential targets of antitumor therapies in view of their increased levels in a wide variety of tumor types. Because of their unique mechanism of action long-term exposure may be a crucial factor, and both will presumably also face us with new toxicology profiles. Related to this, clinical trial design will have to be different from the conventional one used for the development of cytotoxic agents. Early clinical data on the use of MMPI are interesting and support the continued clinical development of this novel class of agents.

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