

Case Report

Roneparstat and Heparanase Inhibition: A New Tool for Cancer Treatment

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Abstract

Heparanase inhibition represents a new and interesting target for addressing cancer as well as other inflammatory-based diseases. This target is still largely underexploited. Heparanase activity releases from the extracellular matrix (ECM) and tumor microenvironment a multitude of heparan sulphate (HS)-bound growth factors, cytokines, chemokines, and enzymes that affect cells and tissue functions such as inflammation, wound healing and tumor invasion. A pro-metastatic and pro-angiogenic role for this enzyme has been widely demonstrated in many primary human tumors since high levels of heparanase correlate with lymph node and distant metastasis, elevated microvessel density and reduced survival of cancer patients. Heparanase is up regulated in many human hematologic and solid tumors. Two HS mimetics, PG545 and Roneparstat, are in active clinical development, the former in solid tumors, and the latter in Multiple Myeloma (MM).

These progresses together with increasing and sound preclinical data suggesting a significant potential for anti-heparanase therapy in many types of tumors, underscores the need to explore the full potential of this novel and safe therapeutic approach. This paper reviews the role of heparanase as novel therapeutic target in cancer and illustrates Roneparstat as a concrete example of such potential.

ABBREVIATIONS

HS: Heparan Sulfate; ECM: Extracellular Matrix; EMT: Endothelial Mesenchymal Transition; MM: Multiple Myeloma; VEGF: Vascular Endothelial Growth Factor; BFGF: Basic Fibroblast Growth Factor; EC: Endothelial Cell; HGF: Hepatocyte Growth Factor; RANKL: Receptor Activator of Nuclear Factor Kappa-B Ligand; IP: Intraperitoneal; IV: Intravenous; Sc: Subcutaneous; BID: Bis in Die

INTRODUCTION

Heparanase is a mammalian endo- β -D-glucuronidase that cleaves heparan sulfate (HS) chains at a limited number of sites. The enzyme is synthesized as a latent 65-kDa precursor that undergoes proteolytic cleavage, yielding 8- and 50 kDa subunits that heterodimerise to form a highly active enzyme. HS cleavage results in remodeling of the extracellular matrix (ECM) as well as in regulating the release of many HS-linked molecules such as growth factors, cytokines and enzymes involved in inflammation, wound healing and tumor invasion. Heparanase up-regulation has been documented in a variety of human tumors correlating in some cases to an increased vascular density and a poor postoperative survival [1,2]. Through activity described above, heparanase influences a multitude of both normal and disease-

related processes - tumor cell growth, invasion and metastasis as well as inflammation, blood coagulation, wound healing, angiogenesis and fibrosis.

HS and the structurally related heparin are present in most animal species. As an analogue of the natural substrate of heparanase, heparin is considered to be a potent inhibitor of heparanase. This activity is attributed to its high affinity interaction with the enzyme and limited degradation, serving as an alternative substrate. Heparin and some chemically modified species of heparin as well as other sulphated polysaccharides that inhibit tumor cell heparanase also inhibit experimental metastasis in animal models, while other related compounds that lack heparanase-inhibiting activity fail to exert an antimetastatic effect.

Considering that, once inactivated, there are no other molecules capable of performing the same function as heparanase, it is evident how this enzyme may be an effective and attractive target for the development of new drugs [3]. This target is however still largely unexploited, and despite different pharmacological structures having been identified as having anti-heparanase activity, only four HS mimetics compounds have successfully made their way to the clinic and two of them, PG545

(Zucero, Australia) and Ronaparstat (Leadiant Biosciences SA), are still in active development. The high and rapidly growing interest combined with the very limited competition makes this new potential target highly attractive. This paper reviews the role of heparanase as a novel therapeutic target in cancer and illustrates Ronaparstat as a concrete example of such potential.

HEPARANASE IN HUMAN DISEASES

The role of heparanase in cancer has attracted a great deal of attention from the scientific community and has been extensively documented. A pro-metastatic and pro-angiogenic role for this enzyme has been widely demonstrated in many primary human tumors because high levels of heparanase correlate with lymph node and distant metastasis, elevated micro vessel density and reduced survival of cancer patients. Data have also been reported that heparanase regulates HS proteoglycan syndecan-1 and promotes its shedding from the cell surface. Shed syndecan-1 in turn controls tumor growth, metastasis and neo-angiogenesis mainly by promoting growth-factor signaling in the tumor's milieu [4]. In addition to its intimate involvement in the egress of cells from the blood stream, heparanase activity releases from the ECM and cancer microenvironment a multitude of HS-bound growth factors, cytokines, chemokines, and enzymes that affect cells and tissue functions [3,5-7].

Heparanase has also been recently reported to play a fundamental role in many other pathologies outside cancer, thus making this pharmacological target of greater interest since it provides a broad range of potential applications. In fact, heparanase over-expression has been observed in several conditions such as cirrhosis, nephrosis, diabetes, fibrosis and other diseases where processes related to cell proliferation and migration as well as mesenchymal transition play a pathogenetic role [3,7,8].

In particular, the role of heparanase in inflammatory disorders has attracted attention and a significant increase in the expression and enzymatic activity of heparanase has been reported in numerous inflammatory conditions, typically associated with degradation of HS and extensive remodeling of the ECM. The ECM heparanase mediated remodeling is thought to facilitate recruitment, extravasation and migration of lymphocytes toward inflammation sites, and lead to activation of innate immune cells. In particular, an involvement of heparanase has been reported in various inflammatory disorders, such as rheumatoid arthritis, hepatitis C infection, chronic and acute pancreatitis, Barrett's esophagus, Crohn's disease and ulcerative colitis, sepsis and others [3,7]. These diseases are typically implicated in initiation of many cancer types in the gastrointestinal tract, pancreas, liver and other tissues, thus further highlighting the role of heparanase as a linking molecule between inflammation and cancer.

The precise mode of heparanase action in inflammatory reactions is complex and its involvement with the different inflammation mediators or players has been reviewed by several Authors extensively [3,7,9]. Recent evidence suggesting heparanase over expression to be related to the endothelial mesenchymal transition (EMT) is also of particular interest. It suggests that heparanase inhibition may be beneficial in counteracting the fibrotic processes which underlay many important degenerative diseases, as liver, kidney or peritoneal fibrosis, where EMT has a well-recognized role [8,10].

HEPARANASE IN CANCER

Immunohistochemistry, in situ hybridization, and real time-PCR analyses reveals that heparanase is up regulated in many human hematologic and solid tumors [3,5,6]. Heparanase is believed to modulate tumor-host microenvironment by degrading and remodeling the extracellular matrix and basement membrane and releasing a number of pro-angiogenic and HS-bound growth factors such as vascular endothelial growth factor (VEGF)-A and basic fibroblast growth factor (bFGF) from the ECM, thus facilitating endothelial cell (EC) migration and proliferation. A critical early event in the angiogenic process is the degradation of the sub endothelial basement membrane, followed by migration toward the angiogenic stimulus [3,6]. In addition to heparanase induced release of growth factors from the ECM, upregulated heparanase expression in cancer cells is also associated with an increase in expression of genes that promote tumor progression including VEGF, HGF and RANKL [3,6,11].

Patients that were diagnosed as 'heparanase positive' exhibited a significantly higher rate of local and distant metastasis as well as a reduced post-operative survival, compared with patients that were diagnosed as 'heparanase-negative'. Furthermore, heparanase up regulation in primary human cancers correlated in most cases with enhanced tumor size and microvessel density, providing clinical support for the pro-angiogenic function of the enzyme [6].

In myeloma patients, heparanase enzymatic activity was elevated in the bone marrow plasma of 86% of patients examined, and gene array analysis showed elevated heparanase expression in 92% of myeloma patients [3,5,6,12,13]. This reported heparanase upregulation was also associated with elevated microvessel density and enhanced expression of the HS proteoglycan syndecan-1 [5,6].

Heparanase is pro-angiogenic while heparanase-dependent regulation of syndecan-1 shedding from the surface of myeloma cells has emerged as highly relevant to disease progression. In fact, syndecan-1 is particularly abundant in myeloma, and whilst surface Syndecan-1 promotes adhesion of myeloma cells and inhibits cell invasion in vitro, high levels of shed Syndecan-1 are found in the serum of some myeloma patients and are associated with poor prognosis [3,4,6,11]. Enhanced heparanase expression and increased heparanase secretion was documented in myeloma cell lines treated with chemotherapy [14,15], thus implicating heparanase as a possible cause of drug resistance [14]. In fact, high heparanase expressing myeloma cells are less susceptible to cytotoxic effects of Bortezomib or Melphalan. Moreover, while Heparanase gene expression was very low in tumor cells isolated from myeloma patients prior to treatment, expression of heparanase was dramatically elevated following chemotherapy [14,15].

HEPARANASE INHIBITION, RONEPARSTAT AND ANTI-CANCER ACTIVITY

Ronaparstat (lab code SST0001) belongs to the 'N-acetyl reduced oxyheparins'. It is a semisynthetic heparin-like polymer that through rational chemical engineering modifications, including a step of reduction-oxidation of a few residues (uronic acid) in addition to a step of hyperacetylation is transformed into a 15–25 kDa glycol-split N-acetyl heparin [16]. Chemical peculiarity

of Roneparstat is therefore a complete N-desulphation followed by a total N-acetylation at the glucosamine residues in addition to 25% glycol-split of a few uronic acid residues (Figure 1). Among anti-heparanase inhibitors belonging to this class of compounds, Roneparstat is the only example that, without altering the nature of native heparin, by introducing other biologically active residues, showed an important reduction of anticoagulant properties associated to a strong inhibition of heparanase activity. A study aimed at investigating the kinetics of heparanase inhibition, through dose-inhibition curves, confirmed the high potency of Roneparstat ($IC_{50} \approx 3$ nM) and highlighted a different behavior of the inhibitor depending upon its concentration, suggesting the existence of multiple protein-ligand interaction modes [17]. To the best of our knowledge, this is a unique and distinctive mechanism among this class of inhibitors; such peculiarity may explain the rather high potency of Roneparstat in inhibiting heparanase and, at the same time, multifaceted vs a single target-drug interaction modality may prospectively make resistance more unlikely to occur. Roneparstat showed a significant anti-Myeloma effect in murine models of multiple myeloma (MM), and caused a significant reduction of subcutaneous growth of different myeloma cell lines, when administered either alone or in combination with Dexamethasone [11].

Moreover, a very significant effect on tumor burden (assessed by kappa protein levels determination and bioluminescence) was observed when Roneparstat (120 mg/kg/day and 60 mg/kg/day, respectively) was combined with Bortezomib (0.5 mg/kg/twice weekly) or Melphalan (1 mg/kg/week) to treat mice bearing a tumor formed by CAG human myeloma cells expressing high levels of heparanase (CAG-HPSE cells). These cells, when injected intravenously, home almost exclusively to bone where they grow aggressively thereby closely mimicking human myeloma [14].

This effect was very pronounced following simultaneous administration of Roneparstat + Bortezomib or Roneparstat

+ Melphalan, though interestingly it was also present when Roneparstat (60 mg/kg/day followed by 120 mg/kg/day) was given as sequential therapy following treatment with Melphalan (2.5 mg/kg/day) [14].

Roneparstat also showed activity in tumors other than MM. In particular, an antitumor effect was reported in lymphomas when given alone or in combination (60 mg/kg/twice daily) with Cyclophosphamide, Rituximab or Bevacizumab [18]. Similarly, a strong inhibitory effect was reported in sarcomas models at 60mg/kg/twice daily, especially when combined with 50 mg/kg/day Irinotecan [19]. Further, an antimetastatic activity was shown in pancreatic and breast cancer as well as in melanoma models [20]. More specifically Roneparstat antimetastatic activity was observed, when delivered ip twice a day at 30mg/kg/day for 3 weeks, to significantly inhibit Panc02 pancreas primary orthotopic tumour growth in C57BL/6J immunocompetent mice and to decrease the number of mesenteric lymph node metastases. Panc02 expressed elevated levels of endogenous heparanase as compared to normal pancreatic tissue. The antimetastatic activity of Roneparstat was observed on B16/BL6 murine melanoma injected IV in syngeneic BALB/c mice (C57BL/6). A single dose of Roneparstat at 60mg/kg/day sc was able to inhibit the number of lung metastases by 63%. Moreover, Roneparstat delivered at 60mg/kg/day bid for 28 days was shown to inhibit bone metastases induced by an intracardiac injection of MDA-MB231 breast carcinoma in BALB/c mice. In addition, continuous administration of 30 mg/kg/day Roneparstat via Alzet pumps has shown activity in Lapatinib resistant breast cancer brain metastasis [21].

RONEPARSTAT IN THE CLINIC

The sound and large preclinical evidence suggested MM as a suitable target for heparanase inhibition and Roneparstat as the drug of choice [3]. Thus Roneparstat started and completed

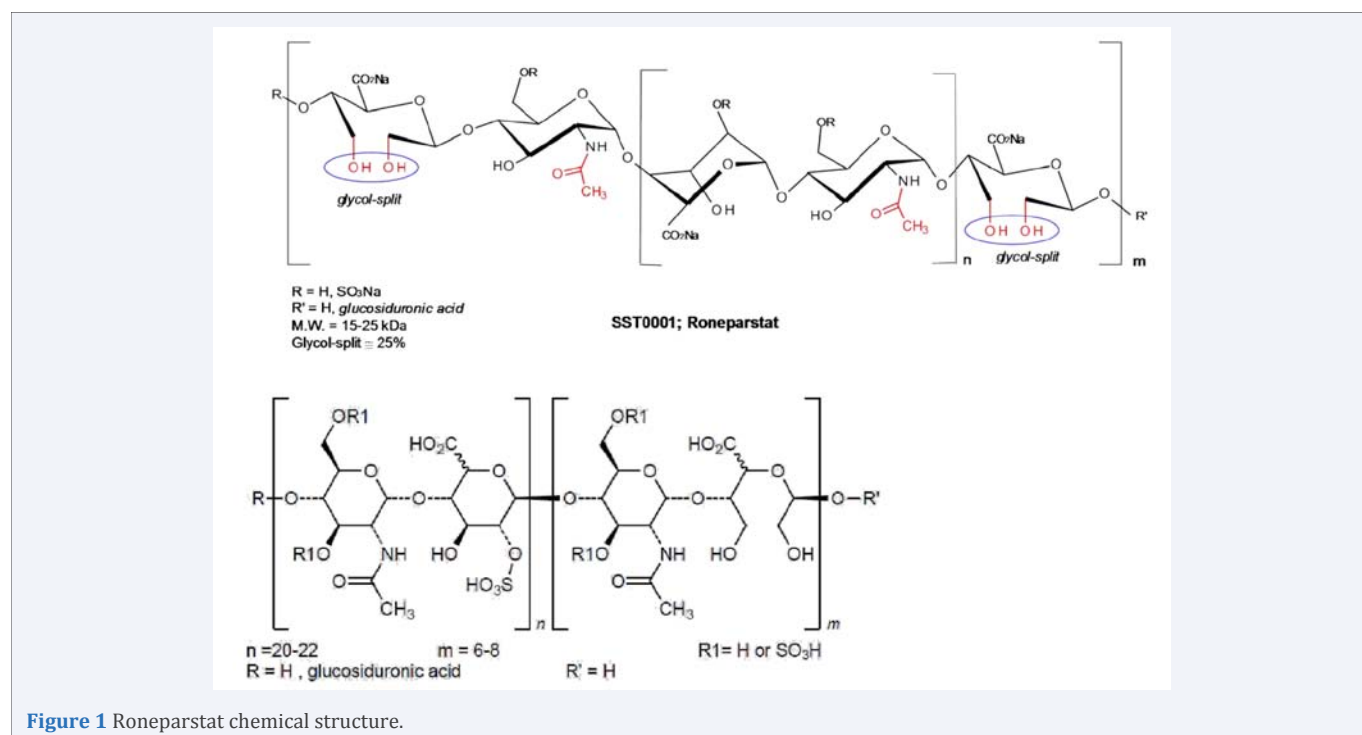


Figure 1 Roneparstat chemical structure.

a phase I, multicenter, international trial in 19 patients with advanced MM [22,23] who exhausted all the available therapeutic options. Results of the trial revealed that Ronaparstat showed excellent tolerability and hints of anti-tumor activities. Moreover, drug plasma levels were measurable and reproducible within the dose range identified as recommended for further phase II studies.

CONCLUSIONS

Heparanase inhibition represents a new and interesting target for addressing cancer as well as other inflammatory-based diseases. However, this target is still largely underexploited. Two HS mimetics, PG545 and Ronaparstat, are currently in active clinical development, the former being the object of an active Phase I study in solid tumors, while the latter has completed a phase I study in MM. These efforts together with increasing and sound preclinical evidence suggesting a significant potential in many tumors, should prompt further studies to explore the full potential of this novel therapeutic approach. Furthermore, in light of the modulating role that heparanase plays in tumor microenvironment thus influencing cell growth and spread as well as attenuating other anti-cancer agents' effect, its inhibition appears as a new, effective and safe target, particularly suitable to enhance combination treatments.

CONFLICT OF INTEREST

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