Role For Chymase in Heart Failure
Angiotensin II-Dependent or -Independent Mechanisms?
Jorma O. Kokkonen, MD, PhD; Ken A. Lindstedt, PhD; Petri T. Kovanen, MD, PhD

One of the major questions regarding the role of angiotensin II (Ang II) in the pathophysiology of heart failure has been whether other enzymes, in addition to angiotensin-converting enzyme (ACE), could contribute to the local production of Ang II in the heart. Specifically, there is controversy as to whether the major Ang II-forming enzyme within the heart is ACE or chymase, a chymotrypsin-like serine protease that is synthesized and stored in the cardiac mast cells and is not affected by ACE inhibitors. It is generally assumed that, during preparation of heart extracts for in vitro experiments, the mast cells present in the myocardial sample are disrupted and, as a result, all the originally intracellular chymase is released into the interstitium. We incubated human heart extracts with Ang I in vitro and in vivo, but the results of these experiments are inconsistent. In vitro experiments with human or animal heart extracts, derived from either normal or failing hearts, have demonstrated unequivocally that the major Ang II-forming enzyme, responsible for 80% to 90% of the Ang II-forming capacity in the heart extracts, is chymase. In striking contrast, experiments in vivo with normal or failing dog hearts have demonstrated that most (>70%) of the Ang II formation can be inhibited by an ACE inhibitor, indicating that, under these conditions, the major Ang II-forming enzyme is ACE. Moreover, in normal human hearts, Zisman et al have demonstrated that, in vivo, the major Ang II-forming enzyme is ACE, as 90% of the Ang II formation could be inhibited by an ACE inhibitor. However, no experiments with intact failing human hearts have been performed.

The obvious explanation for the discrepancy between the in vitro and in vivo studies is that the chymase-mediated Ang II formation is subjected to local regulation, a fact that has been overlooked in the studies performed in vitro. Thus, chymase activity is known to be regulated by at least 2 factors: Those that lead to stimulation and degranulation of mast cells, and those that inhibit chymase activity, i.e., the natural protease inhibitors present in the interstitial fluid.

Firstly, chymase is normally not secreted, but is stored intracellularly in the secretory granules of mast cells, and for chymase to exert its action on the heart, the cardiac mast cells must have been stimulated to degranulate. Mast cells are expected to be stimulated to degranulate in chronic inflammatory states. Indeed, because some of the immunoreactivity of chymase has been found in the extracellular matrix of the failing human heart, at least some of the myocardial mast cells must have degranulated and released chymase. Moreover, the mast cell numbers were found to be increased both in failing human and failing dog hearts. It is important to realize that during preparation of heart extracts for in vitro experiments, the mast cells present in the myocardial sample are all disrupted and, as a result, all the originally intracellular chymase will obtain access to its potential substrates, eg, Ang I. This step corresponds to total degranulation of the mast cells, a situation only approached in vivo in severe allergic anaphylaxis. Thus, in these in vitro tests, the contribution of chymase to Ang II formation is heavily overestimated.

Secondly, the heart chymase-mediated conversion of Ang II takes place in the interstitial fluid. This contains high concentrations of protease inhibitors with the potential to inhibit chymase-mediated Ang-II formation that are lost when heart extracts are prepared. To mimic the actual conditions of angiotensin metabolism in the heart interstitium, we incubated human heart extracts with Ang I in vitro and in vivo, but the results of these experiments are inconsistent. Indeed, because some of the immunoreactivity of chymase has been found in the extracellular matrix of the failing human heart, at least some of the myocardial mast cells must have degranulated and released chymase.

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model of tachycardia-induced heart failure. The use of a dog model of this experimental set-up is appropriate, because dog chymase, like human chymase, has the ability to convert Ang I to Ang II. Thus, positive results obtained with a specific chymase inhibitor in a dog model of heart failure would imply that chymase might have a pathophysiological role in the development of heart failure in humans, as well.

In their study, Matsumoto et al.15 show that dogs, when subjected to ventricular pacing for 3 weeks while receiving vehicle, developed significant interstitial fibrosis and heart failure. In striking contrast, dogs treated with the chymase inhibitor during the pacing period had significantly less collagen deposition and their left ventricular diastolic function was better than that of the dogs that did not receive the inhibitor during the pacing. The authors suggest that the observed antifibrotic effect may depend on a reduction in cardiac Ang II formation. However, treatment with the chymase inhibitor had only a minor inhibitory effect on the cardiac Ang II levels (18%), whereas the collagen volume fraction, representing the level of fibrosis, was significantly reduced (60%). Thus, we can infer that chymase-mediated tissue fibrosis cannot be due solely to its Ang II-forming potential. This finding is in agreement with the concept that ACE, rather than chymase, is the major cardiac Ang II-forming enzyme, and further suggests that the chymase inhibitor-mediated antifibrotic effect occurs independently of a reduction in cardiac Ang II levels.

Interestingly, by using a mast cell-deficient mouse model (W/Wv) and its healthy counterpart, Hara et al.16 have shown that mast cells play a key role in the progression of heart failure. Because mouse chymase is unable to produce Ang II, the adverse effects mediated by the cardiac mast cells, including fibrosis, must have occurred independently of chymase-mediated Ang II formation. As stated by Matsumoto et al.,15 alternative mechanisms exist, for cardiac chymase may also be involved in the generation of active transforming growth factor-β (TGF-β).17,18 another profibrotic molecule.

It seems evident that hemodynamic stress induces the infiltration or proliferation of mast cells in the myocardium, both in humans11 and in experimental animals.12 As observed in the study by Matsumoto et al.,15 the number of chymase-positive mast cells in the myocardium of the vehicle group was significantly increased (13-fold) during the pacing period, whereas their number was much less increased (2.9-fold) in the group receiving chymase inhibitor therapy. Interestingly, it has been shown that mast cell chymase promotes mast cell migration by c-kit ligand (stem cell factor) activation.19 Therefore, it is possible that the observed reduction in mast cell number reflects the effect of the chymase inhibitor on mast cell infiltration. By blocking this infiltration, the chymase inhibitor also indirectly reduces the synthesis and secretion of other mast cell-derived profibrotic molecules, such as tryptase and basic fibroblast growth factor.

As stated above, there are signs of mast cell stimulation and the presence of chymase activity in the extracellular spaces of failing hearts.20 In a hamster model of heart failure, Sukenaga et al.20 have shown that inhibition of chymase activity with NK3201, another potential chymase inhibitor, suppresses mast cell degranulation in vivo. These results show that chymase also participates in mast cell degranulation, suggesting that SUNC8257 may be involved in mast cell stabilization. However, from data presented by Matsumoto et al.,15 it is difficult to judge the amount of chymase present in the extracellular space, i.e., whether or not SUNC8257 inhibits the degree of mast cell stimulation and degranulation.

In summary, the data by Matsumoto et al.15 suggest that most of the potentially harmful effects mediated by chymase are related not to Ang II formation but to other chymase-mediated profibrotic mechanisms, such as promotion of mast cell infiltration, mast cell stimulation, and release of TGF-β. However, we may still be far from understanding the exact cellular and molecular mechanisms by which chymase participates in the progression of heart failure. Therefore, chymase inhibitors may emerge as an important research tool for defining the role of mast cells and mast cell chymase in the pathophysiology of heart failure. To meet with the authors’ suggestion15 that chymase inhibition may become one strategy for preventing cardiac remodeling and the progression of human heart failure, more in vivo experimental work needs to be done and safe and effective chymase inhibitors for human use must be developed.

References


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