Inhibitor-1 is Potential Target for Enhancing Sarcoplasmic Reticulum Ca²⁺ Loading in Failing Hearts

Yasuhiro Ikeda, MD; Masafumi Yano, MD

Dysregulated Ca²⁺ homeostasis is a hallmark of heart failure that is directly linked to cardiac dysfunction and lethal arrhythmia.¹ It is associated with imbalance of kinases/phosphatase activities in the corresponding intracellular microdomains, leading to either hypophosphorylation of phospholamban (PLN)² hyperphosphorylation of ryanodine receptor (RyR)³ or a combination of both in failing cardiomyocytes. It has been increasingly recognized that dysregulation of protein phosphatase 1 (PP1) signaling is associated with the imbalance of kinase/phosphatase in the failing heart.^{3,4} Therefore, PP1 inhibition has been thought of as a potential molecular target for restoring impaired Ca²⁺ cycling mediated by the sarcoplasmic reticulum (SR)⁴. In this regard, the role of inhibitor-1 (I-1), an endogenous PP1 inhibitor, has been drawing attention, not only for understanding the mechanism of increased PP1 activity in failing hearts, but also for its application as a potential therapeutic target.⁵

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I-1 is a ubiquitously expressed acid- and heat-stable cytosolic protein that is primarily rich in skeletal and cardiac muscle.⁶ When I-1's Thr35 residue is phosphorylated by protein kinase A (PKA), I-1 actively inhibits PP1 activity, potentiates PKA phosphorylation, and enhances cardiac contractility. On the other hand, when the Ser67 or Thr75 residues are phosphorylated by protein kinase C α (PKA α)^{7,8} or the Thr35 residue is dephosphorylated by PP2B,⁹ I-1 becomes inactive, resulting in an increase in PP1 activity and suppression of cardiac contractility. Therefore, I-1 is also recognized as an important element of β -adrenergic signaling, as well as Gq-receptor coupled signaling (angiotensin II, endothelin, and α -adrenergic signaling).¹⁰ It has been reported that in heart failure, phosphorylation of I-1 at Thr35 is reduced compared with normal hearts,⁹ and its protein expression level is significantly decreased,¹¹ thereby contributing to impaired β -adrenergic signaling and hence cardiac dysfunction in heart failure.

In this issue, Kawashima et al¹² report a specific effect of I-1 on the SR Ca²⁺ cycling in normal saponin-permeabilized rat cardiomyocytes. They found that administration of I-1

significantly potentiated PKA-mediated SR Ca²⁺ load without affecting Ca²⁺ release function, as assessed by caffeine-induced Ca²⁺ transient and Ca²⁺ spark frequency. The effects of I-1 on SR Ca²⁺ load and Ca²⁺ release function were also characterized as similar to nonspecific chemical inhibition of protein phosphatase by calyculin A. Indeed, there are at least 2 possible mechanisms by which Ca²⁺ spark frequency increased in the permeabilized cardiomyocytes; namely, an increased gating property of the RyR, increased SR Ca²⁺ loading (SR Ca²⁺ content) or a combination of both. SR Ca²⁺ loading seems to be a more important target for I-1 than Ca²⁺ release, because I-1 had no appreciable effect on Ca²⁺ spark frequency and caffeine-induced Ca²⁺ transient after an abrupt inhibition of the SR Ca²⁺ uptake by cyclopiazonic acid, a sarcoendoplasmic ATPase (SERCA2a) inhibitor (Figure 4 in Reference 12). The fact that I-1 had no significant effect on the correlation between intracellular Ca²⁺ concentration and Ca²⁺ spark frequency also suggests that the increase in Ca²⁺ spark frequency is mostly dependent on the SR Ca²⁺ content. In this respect, their findings appear to be consistent with the previously reported findings of Pathak et al¹¹ showing that the constitutive overexpression of I-1 increased cardiac contractility, prevented cardiac hypertrophy and slowed progression of heart failure in a rat pressure-overload-induced heart failure model. Indeed, the constitutive I-1 expression caused an increase in PLN phosphorylation at Ser16, thereby increasing the amplitude of the Ca²⁺ transient and decreasing its decay time. Thus, I-1 is considered to be a potential agent for optimizing SR Ca²⁺ handling in the diseased heart.

However, the interpretation should be carefully made with regard to its therapeutic application. Recent findings by Eli-Armouche et al¹³ contradict the beneficial effect of I-1 on cardiac function. Namely, cardiac overexpression of the full length I-1 rather caused significant cardiac hypertrophy, whereas I-1 knockout mice were resistant to cardiac hypertrophy during chronic administration of high-dose isoproterenol, and moreover, protected from lethal arrhythmia under certain anesthetic conditions. Although it is unclear why the aforementioned 2 types of experiments of I-1 overexpression caused such diverse phenotypes of cardiac hypertrophy, the differences may be partly attributable to the different levels of I-1 expression, lack of I-1 regulatory property in the constitutive I-1 expressed hearts, or a compensatory increase in PP1 catalytic subunits by 3-fold in the full-length I-1 overexpressed hearts. Further study of the in vivo effect of I-1 on SR Ca²⁺ loading should be considered. In particular, clarification of I-1's binding partner may determine the preferential effect of I-1 on SR Ca²⁺ loading, which would be of interest.

On the other hand, the phosphorylation level of RyR at Ser2815 was found to decrease in the I-1 knockout mouse,¹³ suggesting that I-1's effect is also involved in RyR function,

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Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, Ube, Japan

Mailing address: Masafumi Yano, MD, Division of Cardiology, Department of Medicine and Clinical Science, Yamaguchi University Graduate School of Medicine, 1-1-1 Minami-kogushi, Ube 755-8505, Japan. E-mail: yanoma@yamaguchi-u.ac.jp

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which seems inconsistent with the results obtained by Kawashima et al.¹² Further investigation may be required in this aspect.

In addition, it is well known that β -adrenergic stimulation displays a diverse response in association with the receptor downregulation mechanism. As I-1 is also an important element of β -adrenergic receptor signaling, the effect of I-1 on SR Ca²⁺ cycling may differ between the acute and chronic stimulation settings. An experimental design that dissects the acute and chronic effects of β -adrenergic stimulation may be important in future.

In conclusion, Kawashima et al report that I-1 preferentially potentiated the PKA-mediated increase in SR Ca²⁺ loading without appreciable effects on Ca²⁺ release.¹² As PP1, a major element of serine/threonine phosphatases, reportedly plays a critical role in dysregulated Ca²⁺ homeostasis in the failing heart, these findings may provide an important clue to a new therapeutic strategy against heart failure. To suppress PP1 signaling, either small molecular applications or cardiac-specific gene delivery would be effective. The therapeutic usefulness of PP1 inhibition by I-1⁵ or other endogenous inhibitors¹⁴ should be further investigated by dissecting the mechanism and carefully determining potential adverse effects in either heart failure or lethal arrhythmia.

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