ECR European Cardiology Review

REVIEW

Pharmacotherapy

Development of Small-molecule SERCA2a Stimulators: A Novel Class of Ino-lusitropic Agents

Antonio Zaza and Marcella Rocchetti

Department of Biotechnology and Biosciences, University of Milano Bicocca, Milan, Italy

Abstract

Long-term use of modulators of myocardial function has been scaled down because of the partially detrimental effects of currently available agents. Nonetheless, inotropy and lusitropy remain unmet needs in the treatment of chronic heart failure (HF). This underlies the interest in SERCA2a stimulation, a novel approach. This short review describes the identification of SERCA2a stimulating activity in istaroxime, an inotropic compound characterised by its minimal proarrhythmic effects despite its multiple targets, and the development of its derivatives into a novel class of ino-lusitropic agents. The benefits achieved with istaroxime derivatives (versus istaroxime) consist of their selectivity for SERCA2a stimulation and pharmacokinetics suitable for chronic oral administration. Considering the role of SERCA2a depression in HF and the accompanying electrical instability, selective restoration of SERCA2a function provides an appealing approach to HF therapy. Beneficial effects of SERCA2a stimulation by istaroxime have also been recently described in non-cardiac tissues, thus suggesting even broader and unexpected indications for this new class of agents.

Keywords

SERCA2a, PLN antagonist, istaroxime, PST3093, heart failure therapy

Received: 8 November 2024 **Accepted:** 28 February 2025 **Citation:** *European Cardiology Review* 2025;20:e20. **DOI:** https://doi.org/10.15420/ecr.2024.52

Disclosure: The authors have filed EUROPEAN Patent 19202257.2; they have received no payments connected to this patent.

Acknowledgements: Development of istaroxime derivatives was made possible by a close collaboration between the authors (for the physiology component), synthetic chemists (headed by Prof Francesco Peri) and biochemists (headed by Dr Mara Ferrandi). The authors thank Prof F Peri, Dr M Ferrandi, and the respective teams for their pivotal contribution. The studies described in the present review have been supported over the years by research grants from Prassis-Sigma Tau (Italy), CVie Therapeutics (Taiwan) and Windtree Therapeutics (US).

Correspondence: Antonio Zaza, Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, P.za della Scienza 2, 20126 Milano, Italy. E: antonio.zaza@unimib.it

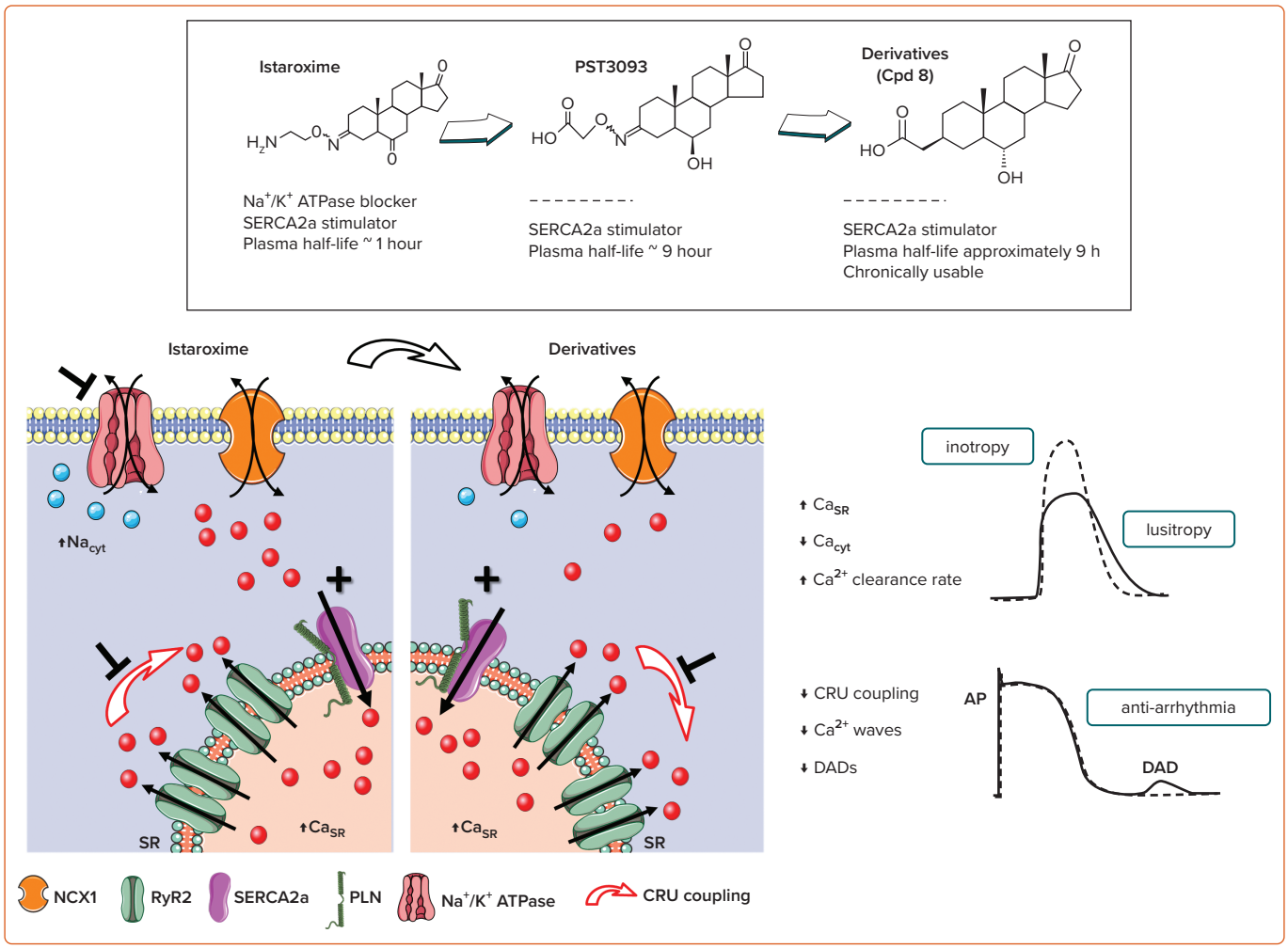
Evidence that the use of positive inotropic agents was associated with an increase in mortality led, in the early 1980s, to a radical change in the approach to chronic heart failure (HF), which switched to the prevention of the maladaptive response (also known as myocardial remodelling) triggered by an initial contractile deficit.[1] Since then, the use of inotropic agents has been confined to the acute phases of the disease or whenever the patient's haemodynamics are severely compromised by pump failure. Nonetheless, it is tempting to speculate that if contractile deficit could be safely countered at its early stage, (mal)adaptive neurohumoral responses would be minimised. Notably, current therapeutic approaches mostly antagonise the cellular effects of such responses, including their adaptive component. This conceivably reduces cardiac adaptability to haemodynamic perturbations, potentially with a negative impact on disease evolution (e.g. by increasing the volumes required to support an adequate cardiac output). This has prompted the search for novel strategies to achieve positive inotropy without the undesired effects, to enable long-term use.

Our involvement in the development of SERCA2a-activating drugs started in 2001, when we were asked by a corporate research laboratory (Prassis-Sigma Tau) to provide a mechanistic interpretation of the surprisingly favourable therapeutic profile (inotropy/toxicity) of a molecule, PST2744, then in their pipeline as a 'digitalis-like' compound.[2] In this brief review, we will revisit the discovery of the unforeseen properties of PST2744, later renamed istaroxime, that are likely to account for its desirable profile, and our journey toward the design and validation of derivatives with improved properties.[3] Because of the novelty of its mechanism of action, PST2744 is a first-in-class agent; indeed, it is the first small-molecule tool for stimulation of sarcoplasmic reticulum (SR) Ca^{2+} ATPase isoform 2a (SERCA2a).[4]

Rationale for SERCA2a Stimulation

SERCA2a is a Ca^{2+}-ATPase located in the membrane of the major intracellular Ca^{2+} store (the SR). Its activity clears approximately 70% of the Ca^{2+} involved in excitation–contraction coupling (Ca^{2+} transient) from the cytosol, and entirely accounts for Ca^{2+} uptake by the SR (internally recycled component of the transient). As such, SERCA2a plays a pivotal role in muscle contraction and relaxation; therefore, its modulation is relevant to inotropy and lusitropy. Partial SERCA2a inhibition by phospholamban (PLN) under basal conditions generates a functional reserve that can be recruited by adrenergic activation through protein kinase A (PKA)-mediated PLN phosphorylation, which reduces PLN affinity for SERCA2a.[5]

Graphical Abstract: Development of Small-molecule SERCA2a Stimulators: A Novel Class of Ino-lusitropic Agents



SERCA2a activity may be severely depressed in HF, mostly because of defective PLN phosphorylation, sometimes associated with downregulation of SERCA2a expression.[6] The concept of therapeutic SERCA2a enhancement in HF has already been explored in a large gene-therapy study.[7] The disappointing outcome of that study is most likely to be due to the low transduction efficiency of the viral vectors in a non-selected population, in whom significant titres of antiviral antibodies are to be expected. The availability of small-molecule SERCA2a stimulators, delivered as normal drugs, would circumvent this problem and the exorbitant costs of gene therapy.

While compensatory mechanisms tend to minimise the effect of SERCA2a dysfunction on force development, this occurs at the expense of altered Ca^{2+} distribution in the intracellular compartments. In other words, even when contractile force can be maintained, higher cytosolic Ca^{2+} levels are required to achieve it if SERCA2a is depressed. This is best summarised by the term 'Ca^{2+} decompartmentation', a condition in which Ca^{2+} localisation is lost, and electrical and mechanical performance is destabilised. Because of the lower energy expenditure of SR Ca^{2+} recycling, Ca^{2+} decompartmentation may also reduce the thermodynamic efficiency of excitation–contraction coupling, as indicated by the effect of SERCA2a stimulation on the cell energy content of diseased myocytes.[8–10] Persistently elevated cytosolic Ca^{2+} levels should also play a pivotal role in myocardial remodelling, as suggested by its reversal by constitutive SERCA2a stimulation.[11,12] However, it has been reported that SERCA2a stimulation fails to prevent activation of the hypertrophy-triggering calcineurin–nuclear factor of activated T-cells pathway, and that a mutation causing marked PLN loss of function (i.e. SERCA2a stimulation) is associated with myocardial remodelling in humans.[13,14] Therefore, whether SERCA2a stimulation may prevent myocardial remodelling is still a matter of debate; differences in the extent of such stimulation and/or ancillary effects (toxicity) of mutant PLN proteins might possibly contribute to the contrasting results.

SERCA2a activation is an important component of myocardial response to catecholamines (and to drugs enhancing cAMP–PKA signalling), which is precisely what β-blocker therapy aims to prevent. Thus, SERCA2a stimulation might appear irrational in the context of HF; nonetheless, this view is probably simplistic. cAMP–PKA signalling simultaneously affects multiple elements of the excitation–contraction machinery, resulting in upregulation of intracellular Ca^{2+} content, electrical instability and hypertrophic gene transcription. In contrast, the main action expected from SERCA2a stimulation alone is to promote Ca^{2+} confinement inside the SR, thus decreasing the cell Ca^{2+} content required to achieve a certain force development.[15] This action may be particularly beneficial in the context of HF, in which SERCA2a depression is a major pathogenetic mechanism. Although Na^+/K^+ pump inhibition may also increase overall cell Ca^{2+} content, it does not promote Ca^{2+} confinement inside the SR and this probably accounts for its proarrhythmic potential.[15]

Overall, restoration of SERCA2a activity seems highly desirable in HF, with expected inotropic, lusitropic and antiarrhythmic benefits. An important

caveat to this view, which generates controversy regarding the benefit of SERCA2a stimulation, is that the resulting increase in SR Ca^{2+} content may promote random opening of Ca^{2+}–SR channels (i.e. ryanodine receptors, RyRs), thus facilitating spontaneous Ca^{2+} release events (SCRs) of proarrhythmic significance. The balance between the advantages and disadvantages of SERCA2a stimulation may conceivably depend on the extent of the initial SERCA2a depression (the larger the better) and on the condition leading to it, with the worst cases theoretically represented by those characterised by primary RyR instability (e.g. RyR mutations). For a detailed discussion of this issue, we refer the reader to the review by Zaza and Rocchetti.[16] Nonetheless, bench evidence and the clinical observation that SERCA2a stimulation by istaroxime is not associated with the proarrhythmic effect expected from concomitant Na^+/K^+ pump inhibition indicates that the advantages may prevail in such a balance.[17–20]

Discovery

PST2744 ((E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride) (*Figure 1*, istaroxime), a derivative of 3,6,17-androstanetrione chemically unrelated to cardiac glycosides, was initially identified as a potential Na^+/K^+ pump inhibitor in a molecular modelling study proposing a new 3D model for the binding of cassaine (a plant alkaloid) at the digitalis receptor site.[21]

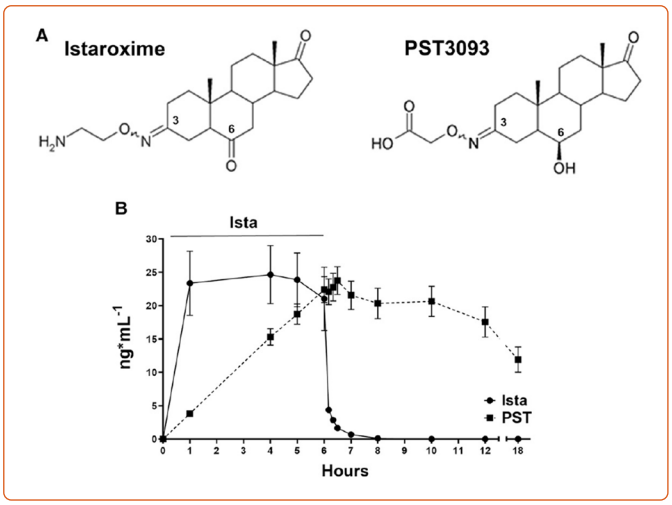
PST2744 was indeed shown to inhibit the Na^+/K^+ pump (digitalis-like action), thus expectedly exerting positive inotropy in animal studies (on guinea pigs).[2] Nonetheless, it was soon noted that, at comparable inotropic effect, proarrhythmia and overall toxicity were significantly lower with PST2744 than with digoxin, the gold standard for digitalis action.[2] Given that proarrhythmia, expected from Na^+/K^+ pump blockade, provides the main rationale for avoiding chronic use of digitalis, such a serendipitous observation triggered interest. As a research group focused on cellular mechanism of arrhythmogenesis, we were then invited to investigate if and how the electrophysiological effect of PST2744 at the cellular level differed from that of digoxin.

The study of cellular electrophysiology involves measurements in isolated myocytes. Therefore, our first concern was to check whether the lower proarrhythmic effect of PST2744 observed in whole animals, could be reproduced in isolated ventricular myocytes. In guinea pig ventricular myocytes, the threshold for aftercontractions (i.e. the indicator of arrhythmia) was at 20% and 60% of twitch amplitude enhancement (i.e. the indicator of inotropy) with digoxin and PST2744, respectively. Overall, in the low equi-inotropic concentration range, there were significantly fewer aftercontractions with PST2744 than with digoxin, and at concentrations lethal with digoxin, no death occurred with PST2744.[2] These encouraging results with PST2744 were further confirmed in dogs with chronic ischaemic HF.[22]

We then proceeded to an extensive comparison of the electrophysiological effects of PST2744 versus digoxin, tested in guinea pig myocytes at equi-inotropic concentrations (4:1).[23] PST2744, but not digoxin, reduced the amplitude of the transient inward current (I_{TI}) following a depolarisation step. This could be explained by either a reduction in the conductance of the Na^+/Ca^{2+} exchanger (NCX), the charge carrier for I_{TI}, or by an acceleration of the Ca^{2+} transient decay, which would blunt the driving force on NCX. NCX conductance was indeed slightly inhibited by PST2744, but the small magnitude of this effect encouraged us to investigate also the latter hypothesis.

This was pursued in the following study, again comparing equi-inotropic

Figure 1: Chemical Structure of Istaroxime and Its Metabolite PST3093 and Pharmacokinetics in Humans



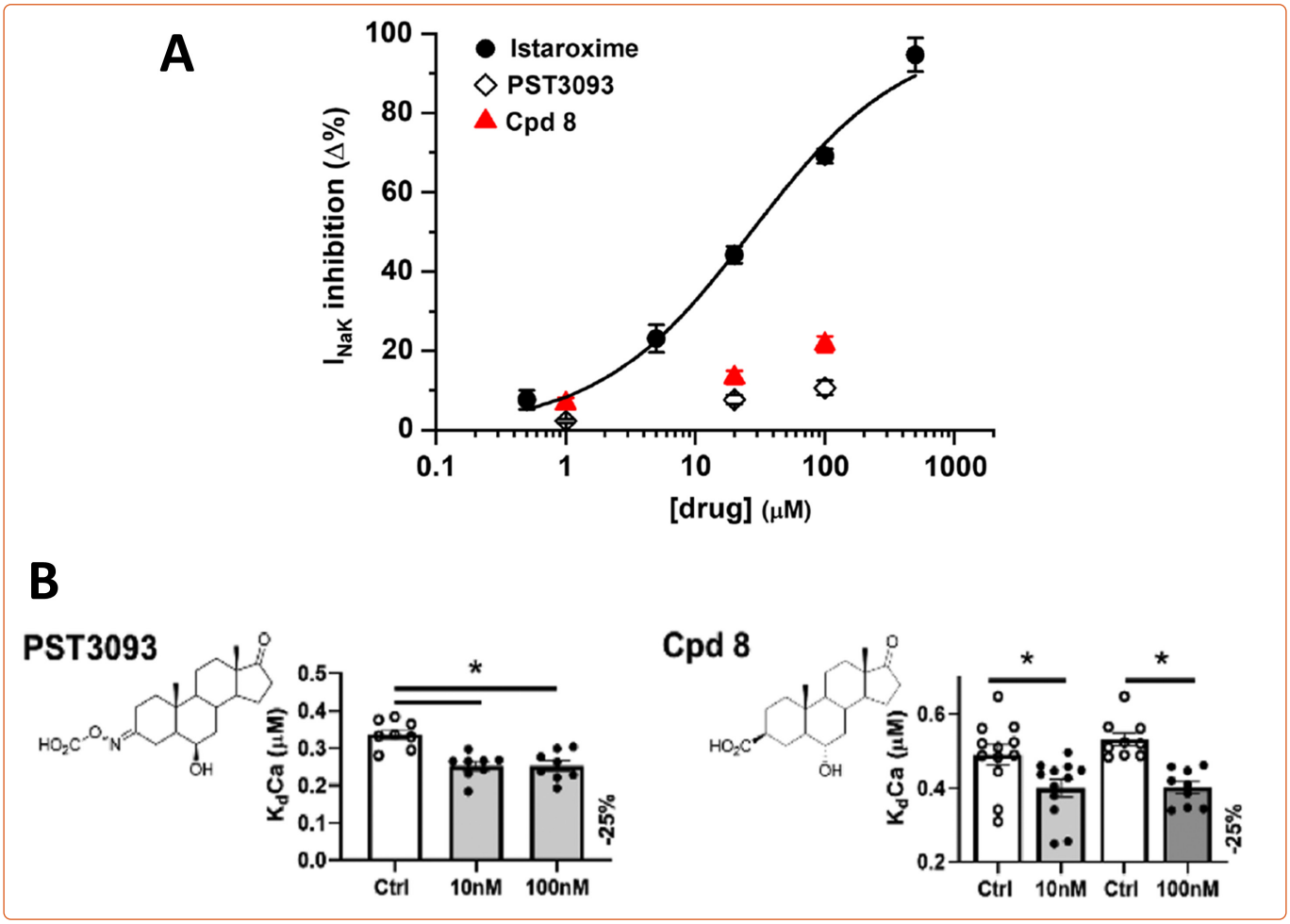
A: PST3093 differs from istaroxime in the replacement of the terminal amino group and reduction of the carbonyl group in position 6. B: The istaroxime plasma level achieved during a 6-hour infusion, quickly subsides upon its discontinuation. PST3093 level slowly increases during istaroxime infusion and is still only partially decayed at 12 h after discontinuation. Source: Arici et al. 2023.[37] Reproduced with permission from Elsevier.

PST2744 and digoxin concentrations in guinea pig myocytes.[24] We first evaluated intracellular Ca^{2+} dynamics under physiological conditions, to find that PST2744 stimulated Ca^{2+} dynamics more than digoxin. This effect was expected from both agents because of the shared modulation of the Na^+/K^+ pump; however, the quantitative difference suggested that PST2744 might be endowed with an extra effect. We then repeated the evaluation under conditions ruling out the Na^+/K^+ pump as the drug target and found that under such conditions PST2744, but not digoxin, robustly enhanced Ca^{2+} uptake by the SR. At this point, SERCA2a was a strong candidate-target to account for the effect specific of PST2744; therefore, we sought the collaboration of Dr Istvan Jona, expert in the evaluation of SERCA2a function (ATPase activity) in a simplified (cell-free) system (microsomes). As a result of the collaboration, we showed that PST2744 was indeed able to enhance the Ca^{2+} dependency of SERCA2a activity.[24] Albeit combined with Na^+/K^+ pump blockade in the case of PST2744, this represented an entirely novel action, of potential relevance to the modulation of myocardial mechanical and electrical functions. Notably, by combining the new findings with those highlighting the better safety profile of PST2744, we hypothesised that SERCA2a stimulation might somehow limit the proarrhythmic effect of Na^+/K^+ pump inhibition. This view, of obvious translational relevance, found strong support in subsequent studies by others, demonstrating that SERCA2a stimulation may prevent proarrhythmia related to SR instability.[17,18]

Translational Applicability and Conceptual Refinement

Although SERCA2 stimulation may also be beneficial for cell function and viability in non-cardiac tissues, the obvious therapeutic target for a SERCA2a stimulator is HF.[25,26] SERCA2a function is generally depressed in remodelled myocardium; hence the concern arose that PST2744 might be unable to enhance SR Ca^{2+} uptake in failing hearts. To address this concern, we tested PST2744 in a guinea pig model of hypertrophy/HF induced by chronic mechanical overload (trans-aortic constriction).[27] As expected from SERCA2a downregulation, SR Ca^{2+} uptake was severely depressed in remodelled myocytes; nonetheless, our concern was proven unjustified by the evidence that PST2744 enhanced SR Ca^{2+} uptake

Figure 2: Modulation of the Na^+/K^+ Pump and SERCA2a by Istaroxime and its Derivatives

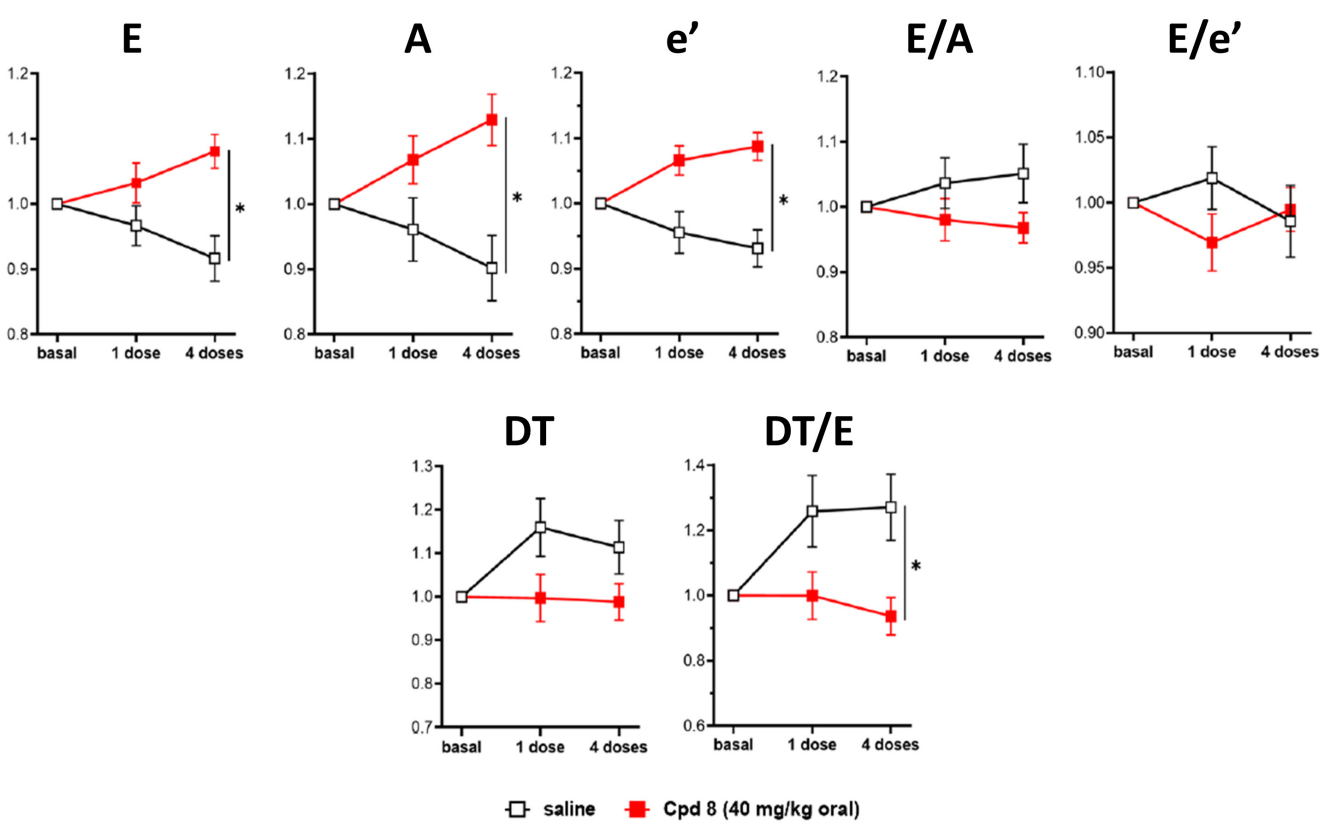


*A: Concentration–response curve for the Na^+/K^+ pump current (I_{NaK}) inhibition by istaroxime, PST3093 and compound (Cpd) 8 (PST3093 derivative) in rat left ventricular myocytes. B: SERCA2a Ca^{2+} affinity (K_dCa) modulation by PST3093 and Cpd 8 in guinea pig microsomal preparations. For technical reasons, separate control groups were used for the two concentrations. *p<0.05. Although the pharmacokinetic properties of the new derivatives have not been tested as yet, like PST3093 they lack the (amino and carbonyl) groups involved in istaroxime catabolism; therefore, they are very likely to have plasma half-lives compatible with oral dosing. Hence, the istaroxime metabolite PST3093 achieves selective SERCA2a stimulation, and its potentially toxic moiety can be replaced without changing the compound's pharmacodynamic profile. SERCA2a = sarcoplasmic reticulum Ca^{2+} ATPase isoform 2a. Source: A: Arici et al. 2024.[39] Reproduced from Springer Nature under a CC BY 4.0 licence. B: Luraghi et al. 2022.[38] Reproduced from ACS Publications under a CC BY 4.0 licence.*

significantly more in remodelled than in normal myocytes.[27] This observation was reproduced in later studies. For example, in rats (intrinsically endowed with strong SERCA2a) the effects of SERCA2a stimulators on SR Ca^{2+} uptake became detectable only after SERCA2a function had been depressed (by streptozotocin [STZ]-induced diastolic dysfunction).[28] We have recently addressed the mechanism underlying this puzzling finding in a computational study. The (as yet unpublished) results indicate that the effect of SERCA2a stimulation on SR Ca^{2+} uptake rate is expectedly maximal at intermediate levels of SERCA2a function (Zaza A, unpublished data). This is because at higher levels, the resulting increase in SR Ca^{2+} content facilitates RyR channel opening, leading to SR Ca^{2+} leak outweighing Ca^{2+} uptake by SERCA2a; this is consistent with the concept of strict homeostatic control of cell Ca^{2+} content.

Homeostatic control of cell Ca^{2+} content predicts that, unlike the multi-target effects of PKA activation (e.g. by β-adrenergic stimulation), selective SERCA2a stimulation should affect cell Ca^{2+} content only marginally. If so, what should we expect from SERCA2a stimulators in terms of overall Ca^{2+} dynamics, inotropy/lusitropy and proarrhythmia? We addressed this question in a study on murine ventricular myocytes, also meant to test for species dependency of istaroxime's effects.[15] We again compared istaroxime with digoxin but focused on the Ca^{2+} distribution between the SR and cytosolic compartments at a given level of Na^+/K^+ pump inhibition and inotropy. The results confirmed the difference between the two agents in terms of SR Ca^{2+} uptake modulation and showed that this difference translated into a diverging compartmentation of intracellular Ca^{2+} during quiescence and sustained stimulation. More specifically, whereas digoxin caused immediate cytosolic Ca^{2+} accumulation in quiescent myocytes, istaroxime did not. During sustained stimulation digoxin shifted the Ca^{2+} transients to higher Ca^{2+} levels without changing their amplitude (i.e. it increased both systolic and diastolic Ca^{2+} similarly); istaroxime increased diastolic Ca^{2+} less than systolic Ca^{2+}, thus achieving a similar Ca^{2+} transients amplitude but at lower diastolic Ca^{2+}. Finally, istaroxime, but not digoxin, slightly increased SR Ca^{2+} content.[15] This suggests that concomitant SERCA2a stimulation may improve subcellular Ca^{2+} compartmentation, thus decreasing the levels of diastolic Ca^{2+} associated with a given inotropy level. Notably, the relationship between SR Ca^{2+} leak and load was similar between the two agents, thus indicating that the improved Ca^{2+} compartmentation by istaroxime was due to SERCA2a stimulation only. Besides the predictable consequence on the ratio between inotropic and lusitropic effects, improved Ca^{2+} compartmentation may have an impact on multiple aspects of myocyte function, including SR stability, energy consumption and, possibly, modulation of hypertrophic transcription.[16]

Figure 3: *In Vivo* Lusitropic Effect of Compound 8 in Streptozotocin Rats (by Echocardiography) after a Single or Repeated Oral Dosing



Normalised indexes of ventricular relaxation (E, e', A, DT) were changed by compound 8 (Cpd 8) to indicate positive lusitropy. Their ratios (E/e' and E/A) were unchanged because both the numerator and denominator were similarly affected by the agent. For most parameters, incremental effects were observed for repeated dosing, thus suggesting a cumulative increase of plasma level with the treatment regimen. DT = deceleration time; STZ = streptozotocin. Source: Arici et al. 2024.[39] Reproduced from Springer Nature under a CC BY 4.0 licence.

From a clinical standpoint, preclinical studies indicate that selective SERCA2a stimulation would provide inotropic and lusitropic effect at a low proarrhythmic risk and, possibly, improve the biology of the failing myocyte. Whereas the ino-lusitropic effect is expectedly smaller than that achieved by PKA activation (e.g. by amine drugs), which entails additional mechanisms, the salient feature of the intervention is probably the ability to restore intracellular Ca^{2+} compartmentation. This compartmentation may be pivotal in preserving electrical stability and in limiting the evolution of cell damage. The conspicuous lack of a proarrhythmic effect of istaroxime in clinical studies (see below) argues against the concern that enhanced SR Ca^{2+} uptake (at least at the level achieved) might be detrimental for electrical stability; it even suggests that SERCA2a stimulation may counter digitalis-like arrhythmogenesis (see Rocchetti and Zaza for further discussion[16]). Clinical data are available only for acute istaroxime treatment; conclusions regarding the long-term risk–benefit ratio of PLN antagonism in the evolution of cardiomyocyte damage (see discussion above) await information from studies on long-term use of SERCA2-selective compounds.

Mechanism of SERCA2a Stimulation

In cardiac, but not skeletal, myocytes, SERCA2a activity is physiologically restrained by an association with PLN, which is, in turn, countered by PLN phosphorylation.[5] The hypothesis that istaroxime might stimulate cardiac SERCA2a by preventing such an interaction was first tested in our 2008 study, which showed istaroxime to be inactive in skeletal muscle microsomes devoid of PLN.[27] Definitive confirmation that istaroxime may stimulate SERCA2a by PLN antagonism came from a further study in microsomes isolated from various sources, showing that the agent physically disrupted the SERCA2a–PLN interaction and its functional effect could be reinstated by SERCA2a–PLN reconstitution.[29] The same study also reported istaroxime effect to be independent of PLN phosphorylation, thus suggesting direct interference with SERCA2a-PLN interaction. PLN antagonism may also account for the persistence of drug efficacy in remodelled myocytes, in which defective PLN phosphorylation (as opposed to reduced SERCA2a expression) prevails in depressing SERCA2a activity.

An istaroxime-induced increase in SERCA2 protein expression was recently described, by immunohistochemistry, in osteocytes of mice with type 2 diabetes.[26] In these cells, istaroxime restored the deranged Ca^{2+} dynamics and, with it, bone mechanoresponsiveness. This was the first study to show an istaroxime mechanism of action upstream of PLN antagonism. Nonetheless, in diabetic osteocytes the restoration of phasic and regulated Ca^{2+} handling led to an overall improvement of gene transcription efficiency, possibly including the SERCA2 gene.[26] Therefore, SERCA2 transcript upregulation might also be secondary to istaroxime-induced increase of SERCA2a function.

Parallel Animal and Clinical Studies

In 2007, a haemodynamic study in dogs with chronic (ischaemic) HF found istaroxime to improve systolic and diastolic function, notably without increasing myocardial O_2 consumption.[30] The authors of the study attributed the effect to SERCA2a stimulation, but forgot to quote where

the notion that istaroxime stimulates SERCA2a came from.[24] While apparently incompatible with an increase in cardiac work (implied by positive inotropy), this observation is consistent with the energy-sparing effect of prevailing Ca^{2+} cycling by SERCA2a, which may improve contraction efficiency (see above).

This preclinical evidence was followed by the evaluation of a 6-hour istaroxime infusion in patients with advanced HF (ejection fraction [EF]<35%).[19] In that study istaroxime, although failing to affect EF, improved several haemodynamic parameters, including systolic blood pressure (SBP). This was not associated with changes in vascular resistances, signs of myocardial damage, or proarrhythmia; however, the istaroxime plasma half-life was deceptively short (<1 hour), thus ruling out oral administration as a treatment modality. Again, the authors attributed istaroxime's effect to SERCA2a stimulation, but to support this view, they surprisingly quoted an article[2] published 3 years before the actual discovery of the action.[24]

A more prolonged (24-hour) istaroxime infusion in HF patients was evaluated in 2020.[20] That recent study confirmed istaroxime efficacy, with more clear-cut haemodynamic effects and reduction of biochemical markers of chamber dilation, again in the absence of proarrhythmia or signs of myocardial damage. Blood samples for pharmacokinetic analysis were collected from a subset of patients, but plasma drug levels were not reported.

The hypothesis that inotropy by a non-adrenergic intervention may be safe and effective in haemodynamic support of the subset of HF patients in pre-cardiogenic shock (SBP<90 mmHg) was recently tested. In that pilot multicentre study (SEISMiC trial), 24-hour istaroxime infusion increased SBP and improved several echocardiographic indexes without major side effects.[31] Notably, SBP and cardiac index increased concomitantly, an effect unique to istaroxime (i.e. never observed for other IV drugs in this class of patients). Even considering that istaroxime is also endowed with a digitalis-like action, this observation points to the potential benefit of non-adrenergic SERCA2a stimulation. Based on such positive findings, a further trial (SEISMiC-B) of an increased istaroxime infusion (to 60 hours) has been announced.[32]

Further articles supporting the positive haemodynamic effects of istaroxime in acute HF patients in general were published thereafter, as summarised in two recent meta-analyses.[33,34] An exception to this view comes from the failure of istaroxime to improve diastolic indexes in a small sample of patients with HF with preserved EF during exercise.[35] Although this observation may seem surprising in light of the primarily lusitropic effect of SERCA2a stimulation, structural components (collagen, titin, etc.) may prevail over dysregulation of Ca^{2+} handling in hampering relaxation of hypertrophied hearts.[36]

Development of Istaroxime Derivatives

As noted in the introduction, besides their use in infusions in acute HF, low-toxicity inotropic drugs might be used in the long term for contractile support at early disease stages. This requires repetitive oral dosing at convenient intervals, for which istaroxime is ill-suited because of its very short plasma half-life. A further problem with long-term istaroxime treatment may be the presence of an oxime moiety, which is considered as potentially genotoxic in long-term use. Finding a solution to these two problems was the main motive for our subsequent endeavour to suitably modify the istaroxime molecule. Such an endeavour was carried out through close collaboration with biochemists and synthetic chemists in a departmental joint-laboratory initiative.

Istaroxime is quickly metabolised in the liver by oxidative deamination of the primary amino group and reduction of the carbonyl in position 6, resulting in a terminal metabolite named PST3093. Pharmacokinetic analysis of the human blood samples collected in the 2020 clinical study confirmed the very short half-life of istaroxime, but detected a very large accumulation of PST3093, which soon exceeded the istaroxime plasma levels and decayed with a half-life >8 hours (*Figure 1*).[20,37]

This serendipitous observation led us to consider the possibility that PST3093 might retain istaroxime's effects, thus affording a means to achieve them chronically, by oral dosing. As an experimental model to test this hypothesis we chose the rat; however, preliminary experiments showed that healthy rat myocytes were insensitive even to istaroxime. Therefore, following the idea that depressed SERCA2a is more responsive to stimulators, we switched to a diabetic rat model (STZ-induced diabetes). In diabetic myocytes, SERCA2a activity was indeed severely depressed and PST3093 almost completely recovered it, as well as SR Ca^{2+} uptake, to the levels observed in healthy myocytes. PST3093 was also found to restore *in vivo* haemodynamics in the diabetic model, thus demonstrating the pivotal role of SERCA2a downregulation in the dysfunction of diabetic hearts.[37] At concentrations corresponding to the observed plasma levels, PST3093 efficacy was similar to that of istaroxime. This suggests that the metabolite might actually account for the haemodynamic effects of the parent compound observed in patients.[20] As for istaroxime, SERCA2a modulation by PST3093 was found to depend on the presence of PLN, thus qualifying the compound as a PLN antagonist. We then proceeded to evaluate modulation of the Na^+/K^+ pump and found that PST3093 did not share the inhibitory effect of istaroxime (*Figure 2*).[37] Except for the persistence of a minor shortening of repolarisation, PST3093 was also found to be devoid of electrophysiological effects and toxicity, consistent with the safety of istaroxime infusion, during which the metabolite was present at high concentrations.[20,37] To summarise, at variance with its parent compound, PST3093 was found to be a pure SERCA2a stimulator, with an interesting pharmacodynamic profile, and was an excellent starting point for the design of an entirely novel class of ino-lusitropic agents.

PST3093 still retains the oxime moiety, which would hinder its chronic therapeutic use. To address this problem, we started a collaboration with a synthetic chemistry group in our department, which yielded a series of new PST3093 derivatives devoid of the oxime moiety.[38] The oxime C double bond was replaced with either an alkene group, or with a saturated C-C bond in the β configuration. The new molecules were screened for their stimulation of SERCA2a activity in microsomes, highlighting the essential role of the saturated C-C bond in preserving the effect. Two leads were further validated for modulation of SR Ca^{2+} uptake and electrophysiology in myocytes of diabetic rats and in the same model for *in vivo* haemodynamic effects.[38] Similar to the parent compound, the derivatives failed to block the Na^+/K^+ pump (pure SERCA2a activators; *Figure 2*) and their effect depended on the presence of PLN (PLN antagonists). Moreover, a derivative of PST3093 (compound 8), still devoid of a Na^+/K^+ pump blocking effect (*Figure 2*), was shown to reverse STZ-induced diastolic dysfunction *in vivo* by oral administration (*Figure 3*), thus providing a mechanism-based lusitropic treatment for chronic HF.[39]

Conclusion

Insightful interpretation of the favourable therapeutic profile of PST2744 (now known as istaroxime), a putative digitalis-like compound, has disclosed the possibility of achieving stimulation of SERCA2a function by a small molecule. This represents a significant advancement with respect

to SERCA2a modulation by gene-based approaches, and establishes a novel class of ino-lusitropic drugs. Subsequent refinement of the parent compound has yielded derivatives with strong SERCA2a selectivity and long plasma half-lives, suitable for long-term use. While the safety and haemodynamic efficacy of istaroxime in the acute HF setting have been confirmed by several independent trials, the long-term use of its derivatives remains to be tested in the clinical setting. Intriguingly, evidence of the biological efficacy of istaroxime in extra-cardiac targets is emerging, thus suggesting an unforeseen benefit of SERCA stimulation in restoring the function of multiple organs.[26] ❑

1. Xu Y, Tuteja D, Zhang Z, et al. Molecular identification and functional roles of a Ca(2+)-activated K+ channel in human and mouse hearts. *J Biol Chem* 2003;278:49085–94. https://doi.org/10.1074/jbc.M307508200; PMID: 13679367.
2. Micheletti R, Mattera GG, Rocchetti M, et al. Pharmacological profile of the novel inotropic agent (E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride (PST2744). *J Pharmacol Exp Ther* 2002;303:592–600. https://doi.org/10.1124/jpet.102.038331; PMID: 12388640.
3. Ghali JK, Smith WB, Torre-Amione G, et al. A phase 1–2 dose-escalating study evaluating the safety and tolerability of istaroxime and specific effects on electrocardiographic and hemodynamic parameters in patients with chronic heart failure with reduced systolic function. *Am J Cardiol* 2007;99:47A–56A. https://doi.org/10.1016/j.amjcard.2006.09.006; PMID: 17239705.
4. Khan H, Metra M, Blair JE, et al. Istaroxime, a first in class new chemical entity exhibiting SERCA-2 activation and Na-K-ATPase inhibition: a new promising treatment for acute heart failure syndromes? *Heart Fail Rev* 2009;14:277–87. https://doi.org/10.1007/s10741-009-9136-z; PMID: 19238540.
5. Bers DM. *Excitation–Contraction Coupling and Cardiac Contractile Force*. 2nd ed. Dordrecht, Netherlands: Springer, 2001. https://doi.org/10.1007/978-94-010-0658-3.
6. Lipskaia L, Chemaly ER, Hadri L, et al. Sarcoplasmic reticulum Ca(2+) ATPase as a therapeutic target for heart failure. *Expert Opin Biol Ther* 2010;10:29–41. https://doi.org/10.1517/14712590903321462; PMID: 20078230.
7. Greenberg B, Butler J, Felker GM, et al. Calcium upregulation by percutaneous administration of gene therapy in patients with cardiac disease (CUPID 2): a randomised, multinational, double-blind, placebo-controlled, phase 2b trial. *Lancet* 2016;387:1178–86. https://doi.org/10.1016/S0140-6736(16)00082-9; PMID: 26803443.
8. Bers DM. Calcium fluxes involved in control of cardiac myocyte contraction. *Circ Res* 2000;87:275–81. https://doi.org/10.1161/01.res.87.4.275; PMID: 10948060.
9. Sakata S, Lebeche D, Sakata N, et al. Restoration of mechanical and energetic function in failing aortic-banded rat hearts by gene transfer of calcium cycling proteins. *J Mol Cell Cardiol* 2007;42:852–61. https://doi.org/10.1016/j.yjmcc.2007.01.003; PMID: 17300800.
10. O'Donnell JM, Pound K, Xu X, Lewandowski ED. SERCA1 expression enhances the metabolic efficiency of improved contractility in post-ischemic heart. *J Mol Cell Cardiol* 2009;47:614–21. https://doi.org/10.1016/j.yjmcc.2009.08.031; PMID: 19744494.
11. Prestle J, Quinn FR, Smith GL. Ca(2+)-handling proteins and heart failure: novel molecular targets? *Curr Med Chem* 2003;10:967–81. https://doi.org/10.2174/0929867033457656; PMID: 12678683.
12. Hoshijima M, Ikeda Y, Iwanaga Y, et al. Chronic suppression of heart-failure progression by a pseudophosphorylated mutant of phospholamban via in vivo cardiac rAAV gene delivery. *Nat Med* 2002;8:864–71. https://doi.org/10.1038/nm739; PMID: 12134142.
13. Louch WE, Vangheluwe P, Bito V, et al. Phospholamban ablation in hearts expressing the high affinity SERCA2b isoform normalizes global Ca(2)(+) homeostasis but not Ca(2)(+)-dependent hypertrophic signaling. *Am J Physiol Heart Circ Physiol* 2012;302:H2574–82. https://doi.org/10.1152/ajpheart.01166.2011; PMID: 22505640.
14. Haghighi K, Kolokathis F, Pater L, et al. Human phospholamban null results in lethal dilated cardiomyopathy revealing a critical difference between mouse and human. *J Clin Invest* 2003;111:869–76. https://doi.org/10.1172/JCI17892; PMID: 12639993.
15. Alemanni M, Rocchetti M, Re D, Zaza A. Role and mechanism of subcellular Ca2+ distribution in the action of two inotropic agents with different toxicity. *J Mol Cell Cardiol* 2011;50:910–18. https://doi.org/10.1016/j.yjmcc.2011.02.008; PMID: 21354172.
16. Zaza A, Rocchetti M. Calcium store stability as an antiarrhythmic endpoint. *Curr Pharm Des* 2015;21:1053–61. https://doi.org/10.2174/1381612820666141029100650; PMID: 25354186.
17. Davia K, Bernobich E, Ranu HK, et al. SERCA2A overexpression decreases the incidence of aftercontractions in adult rabbit ventricular myocytes. *J Mol Cell Cardiol* 2001;33:1005–15. https://doi.org/10.1006/jmcc.2001.1368; PMID: 11343422.
18. Bai Y, Jones PP, Guo J, et al. Phospholamban knockout breaks arrhythmogenic Ca2+ waves and suppresses catecholaminergic polymorphic ventricular tachycardia in mice. *Circ Res* 2013;113:517–26. https://doi.org/10.1161/CIRCRESAHA.113.301678; PMID: 23856523.
19. Gheorghiade M, Blair JE, Filippatos GS, et al. Hemodynamic, echocardiographic, and neurohormonal effects of istaroxime, a novel intravenous inotropic and lusitropic agent: a randomized controlled trial in patients hospitalized with heart failure. *J Am Coll Cardiol* 2008;51:2276–85. https://doi.org/10.1016/j.jacc.2008.03.015; PMID: 18534276.
20. Carubelli V, Zhang Y, Metra M, et al. Treatment with 24 hour istaroxime infusion in patients hospitalised for acute heart failure: a randomised, placebo-controlled trial. *Eur J Heart Fail* 2020;22:1684–93. https://doi.org/10.1002/ejhf.1743; PMID: 31975496.
21. De Munari S, Barassi P, Cerri A, et al. A new approach to the design of novel inhibitors of Na+,K+-ATPase: 17 alpha-substituted seco-D 5beta-androstane as cassaine analogues. *J Med Chem* 1998;41:3033–40. https://doi.org/10.1021/jm980108d; PMID: 9685243.
22. Adamson PB, Vanoli E, Mattera GG, et al. Hemodynamic effects of a new inotropic compound, PST-2744, in dogs with chronic ischemic heart failure. *J Cardiovasc Pharmacol* 2003;42:169–73. https://doi.org/10.1097/00005344-200308000-00003; PMID: 12883318.
23. Rocchetti M, Besana A, Mostacciuolo G, et al. Diverse toxicity associated with cardiac Na+/K+ pump inhibition: evaluation of electrophysiological mechanisms. *J Pharmacol Exp Ther* 2003;305:765–71. https://doi.org/10.1124/jpet.102.047696; PMID: 12606646.
24. Rocchetti M, Besana A, Mostacciuolo G, et al. Modulation of sarcoplasmic reticulum function by Na+/K+ pump inhibitors with different toxicity: digoxin and PST2744 [(E,Z)-3-((2-aminoethoxy)imino)androstane-6,17-dione hydrochloride]. *J Pharmacol Exp Ther* 2005;313:207–15. https://doi.org/10.1124/jpet.104.077933; PMID: 15576469.
25. Li L, Zeng X, Liu Z, et al. Mesenchymal stromal cells protect hepatocytes from lipotoxicity through alleviation of endoplasmic reticulum stress by restoring SERCA activity. *J Cell Mol Med* 2021;25:2976–93. https://doi.org/10.1111/jcmm.16338; PMID: 33591626.
26. Shao X, Tian Y, Liu J, et al. Rescuing SERCA2 pump deficiency improves bone mechano-responsiveness in type 2 diabetes by shaping osteocyte calcium dynamics. *Nat Commun* 2024;15:890. https://doi.org/10.1038/s41467-024-45023-6; PMID: 38291059.
27. Rocchetti M, Alemanni M, Mostacciuolo G, et al. Modulation of sarcoplasmic reticulum function by PST2744 [istaroxime; (E,Z)-3-((2-aminoethoxy)imino) androstane-6,17-dione hydrochloride)] in a pressure-overload heart failure model. *J Pharmacol Exp Ther* 2008;326:957–65. https://doi.org/10.1124/jpet.108.138701; PMID: 18539651.
28. Torre E, Arici M, Lodrini AM, et al. SERCA2a stimulation by istaroxime improves intracellular Ca2+ handling and diastolic dysfunction in a model of diabetic cardiomyopathy. *Cardiovasc Res* 2022;118:1020–32. https://doi.org/10.1093/cvr/cvab123; PMID: 33792692.
29. Ferrandi M, Barassi P, Tadini-Buoninsegni F, et al. Istaroxime stimulates SERCA2a and accelerates calcium cycling in heart failure by relieving phospholamban inhibition. *Br J Pharmacol* 2013;169:1849–61. https://doi.org/10.1111/bph.12278; PMID: 23763364.
30. Sabbah HN, Imai M, Cowart D, et al. Hemodynamic properties of a new-generation positive luso-inotropic agent for the acute treatment of advanced heart failure. *Am J Cardiol* 2007;99:41A–6A. https://doi.org/10.1016/j.amjcard.2006.09.005; PMID: 17239704.
31. Metra M, Chioncel O, Cotter G, et al. Safety and efficacy of istaroxime in patients with acute heart failure-related pre-cardiogenic shock: a multicentre, randomized, double-blind, placebo-controlled, parallel group study (SEISMiC). *Eur J Heart Fail* 2022;24:1967–77. https://doi.org/10.1002/ejhf.2629; PMID: 35867804.
32. Biegus J, Mebazaa A, Metra M, et al. Safety and efficacy of up to 60 h of IV istaroxime in pre-cardiogenic shock patients: design of the SEISMiC trial. *ESC Heart Fail* 2025;12:189–98. https://doi.org/10.1002/ehf2.15102; PMID: 39375885.
33. Abuelazm M, Ali S, AlBarakat MM, et al. Istaroxime for patients with acute heart failure: a systematic review and meta-analysis of randomized controlled trials. *Diseases* 2023;11:183. https://doi.org/10.3390/diseases11040183; PMID: 38131989.
34. Khalid Khan S, Rawat A, Khan Z, et al. Safety and efficacy of istaroxime in patients with acute heart failure: a meta-analysis of randomized controlled trials. *Cureus* 2023;15:e41084. https://doi.org/10.7759/cureus.41084; PMID: 37519574.
35. Sarma S, MacNamara JP, Hieda M, et al. SERCA2a agonist effects on cardiac performance during exercise in heart failure with preserved ejection fraction. *JACC Heart Fail* 2023;11:760–71. https://doi.org/10.1016/j.jchf.2023.02.006; PMID: 37086245.
36. Roe ÅT, Aronsen JM, Skardal K, et al. Increased passive stiffness promotes diastolic dysfunction despite improved Ca2+ handling during left ventricular concentric hypertrophy. *Cardiovasc Res* 2017;113:1161–72. https://doi.org/10.1093/cvr/cvx087; PMID: 28472418.
37. Arici M, Ferrandi M, Barassi P, et al. Istaroxime metabolite PST3093 selectively stimulates SERCA2a and reverses disease-induced changes in cardiac function. *J Pharmacol Exp Ther* 2023;384:231–44. https://doi.org/10.1124/jpet.122.001335; PMID: 36153005.
38. Luraghi A, Ferrandi M, Barassi P, et al. Highly selective SERCA2a activators: preclinical development of a congeneric group of first-in-class drug leads against heart failure. *J Med Chem* 2022;65:7324–33. https://doi.org/10.1021/acs.jmedchem.2c00347; PMID: 35580334.
39. Arici M, Hsu SC, Ferrandi M, et al. Selective SERCA2a activator as a candidate for chronic heart failure therapy. *J Transl Med* 2024;22:77. https://doi.org/10.1186/s12967-024-04874-9; PMID: 38243248.