Free Radical Biology and Medicine 74 (2014) 210–221

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original Contribution

# Comparison of various iron chelators and prochelators as protective agents against cardiomyocyte oxidative injury

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ARTICLE INFO

*Article history:*
Received 4 March 2014
Received in revised form
17 June 2014
Accepted 24 June 2014
Available online 30 June 2014

*Keywords:*
Iron chelation
Deferasirox
ICL670A
Salicylaldehyde isonicotinoyl hydrazone
Prochelator
BSIH
Free radicals

ABSTRACT

Oxidative stress is a common denominator of numerous cardiovascular disorders. Free cellular iron catalyzes the formation of highly toxic hydroxyl radicals, and iron chelation may thus be an effective therapeutic approach. However, using classical iron chelators in diseases without iron overload poses risks that necessitate more advanced approaches, such as prochelators that are activated to chelate iron only under disease-specific oxidative stress conditions. In this study, three cell-membrane-permeable iron chelators (clinically used deferasirox and experimental SIH and HAPI) and five boronate-masked prochelator analogs were evaluated for their ability to protect cardiac cells against oxidative injury induced by hydrogen peroxide. Whereas the deferasirox-derived agents TIP and TRA-IMM displayed negligible protection and even considerable toxicity, the aroylhydrazone prochelators BHAPI and BSIH-PD provided significant cytoprotection and displayed lower toxicity after prolonged cellular exposure compared to their parent chelators HAPI and SIH, respectively. Overall, the most favorable properties in terms of protective efficiency and low inherent cytotoxicity were observed with the aroylhydrazone prochelator BSIH. BSIH efficiently protected both H9c2 rat cardiomyoblast-derived cells and isolated primary rat cardiomyocytes against hydrogen peroxide-induced mitochondrial and lysosomal dysregulation and cell death. At the same time, BSIH was nontoxic at concentrations up to its solubility limit (600 μM) and in 72-h incubation. Hence, BSIH merits further investigation for prevention and/or treatment of cardiovascular disorders associated with a known (or presumed) component of oxidative stress.



Iron (Fe) is an essential element for all mammalian cells and is the most plentiful transition metal in the human body. Because of its flexibility as both electron donor and electron acceptor, Fe is a key cofactor of proteins critical to such basic cellular processes as oxygen transport and storage; electron transport; cellular respiration; DNA, RNA, and protein synthesis; cellular proliferation and differentiation; and regulation of gene expression [1]. The importance of maintaining Fe homeostasis is highlighted by the fact that an imbalance in either direction causes pathophysiological disorders: deficiency leads to anemia and overload leads to tissue damage and organ failure as a result of Fe-promoted oxidative stress [1]. Redox-active Fe species efficiently catalyze the Haber–Weiss reaction of superoxide radical and hydrogen peroxide ($H_2O_2$) to yield hydroxyl radicals—the most reactive and toxic form of reactive oxygen species (ROS) [2–4].

Considering the propensity of free Fe to mediate oxidative injury, cells acquire, transport, and store Fe by employing dedicated proteins that maintain the intracellular labile and redox-active Fe pool at an appropriate level. Nevertheless, owing to the lack of effective body Fe excretion, excess body Fe can accumulate in some diseases with overactive Fe absorption and/or regular blood transfusion therapy, such as β-thalassemia major [1]. In addition, local Fe-mediated oxidative injury may occur even without systemic Fe overload [2–4]. Reactive oxygen or nitrogen

*Abbreviations:* BHAPI, (*E*)-*N*′-[1-(2-((4-(4,4,5,5-tetramethyl-1,2,3-dioxoborolan-2-yl)benzyl)oxy)phenyl)ethylidene] isonicotinohydrazide; BSIH, (*E*)-*N*′-[2-(4,4,5,5-tetramethyl[1,2,3]dioxaborolan-2-yl)benzylidene] isonicotinohydrazide; BSIH-PD, (*E*)-*N*′-[2-(3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,2,3]dioxaborolan-2-yl)benzylidene] isonicotinohydrazide; DFO, desferrioxamine; DMSO, dimethyl sulfoxide; FAC, ferric ammonium citrate; FAS, ferrous diammonium sulfate; HAPI, (*E*)-*N*′-[1-(2-hydroxyphenyl)ethyliden] isonicotinohydrazide; ICL670A, 4-[(3*Z*,5*E*)-3,5-bis-(6-oxo-1-cyclohexa-2,4-dienylidene)-1,2,4-trizolidin-1-yl] benzoic acid; L1, deferiprone; ROS, reactive oxygen species; SIH, (*E*)-*N*′-(2-hydroxybezylidene) isonicotinohydrazide; TIP, 4-[5-(2-((4-boronobenzyl)oxy)phenyl)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl]benzoic acid; TRA-IMM, methyl-4-[3-(2-hydroxyphenyl)-5-(2-((4-(4,4,5,5-tetramethyl-1,2,3-dioxoborolan-2-yl)benzyl)oxy)-phenyl)-1*H*-1,2,4-triazol-1-yl] benzoate

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http://dx.doi.org/10.1016/j.freeradbiomed.2014.06.019
0891-5849/

species may severely deregulate cellular Fe homeostasis [5]. For example, high cellular levels of superoxide and peroxide species, as well as low pH (both of which occur during myocardial ischemia/reperfusion injury), cause Fe release from its storage proteins, thereby increasing the cytosolic pool of labile Fe and exacerbating the production of deleterious ROS [6]. Furthermore, redox-active Fe may also promote the formation of ROS within mitochondria and lysosomes [7] and thus propagate the vicious cycle of oxidative injury.

Oxidative stress is a common denominator of a wide range of cardiovascular disorders. These include ischemia/reperfusion injury, cardiac arrhythmias, congestive heart failure, myocarditis, atherosclerosis, hypertension, and the cardiotoxicity of redox-cycling drugs [8]. However, conventional treatment of cardiovascular diseases still lacks the use of antioxidants owing to mostly negative study outcomes [9,10]. Unfortunately, efforts to mitigate oxidative stress have so far primarily focused on administering ROS scavengers, rather than on actual prevention of ROS production. This can be achieved with Fe chelators, i.e., agents that form redox-inactive complexes with Fe and block the Fe-dependent and site-specific production of hydroxyl radicals [11–13]. Fe chelators have been tested in numerous cardiovascular diseases [14,15]. However, progress in this area has been hindered by the lack of suitable ligands. Probably because of its commercial availability, most studies have employed desferrioxamine (DFO), a bacterial siderophore clinically used for lowering the body Fe burden in disorders such as β-thalassemia [13]. Unfortunately, this hydrophilic chelator suffers from poor plasma membrane permeativity, resulting in limited access to intracellular labile iron pools [13]. Previous results from our laboratory as well as those of other groups have shown promising cardioprotective potential (both in vitro and in vivo) of various small-molecule and lipophilic Fe chelators, both clinically used agents such as deferasirox (ICL670A; 4-[(3*Z*,5*E*)-3,5-bis-(6-oxo-1-cyclohexa-2,4-dienylidene)-1,2,4-trizolidin-1-yl] benzoic acid) and deferiprone (L1) and experimental aroylhydrazone chelators including salicylaldehyde isonicotinoyl hydrazone (SIH; (*E*)-*N*′-(2-hydroxybezylidene) isonicotinohydrazide;) and its novel analog with improved hydrolytic stability HAPI ((*E*)-*N*′-[1-(2-hydroxyphenyl)ethyliden] isonicotinohydrazide) [16–22].

The use of Fe-chelating agents in states without systemic Fe overload, however, bears a toxicity risk due to chelator-induced deregulation of physiological Fe homeostasis [12]. Hence, for diseases associated with localized Fe-mediated oxidative stress without systemic Fe overload, an optimal chelator should be capable of permeating cell membranes, but preferentially sequestering only those Fe ions that are causing damage without withholding Fe or other metals from metalloproteins or inducing systemic metal excretion. To this end, novel boronate-masked prochelators were recently introduced [23]. These prochelators are designed not to bind metal ions unless the protective mask is conditionally removed by $H_2O_2$ to reveal an active chelator capable of suppressing Fe-mediated hydroxyl radical generation (Fig. 1).

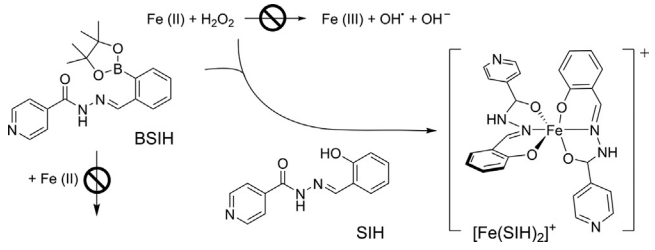


**Fig. 1.** Concept of oxidative stress-activated iron chelation. The conversion of the prochelator BSIH to an active chelating agent, SIH, by reaction with $H_2O_2$ is shown.

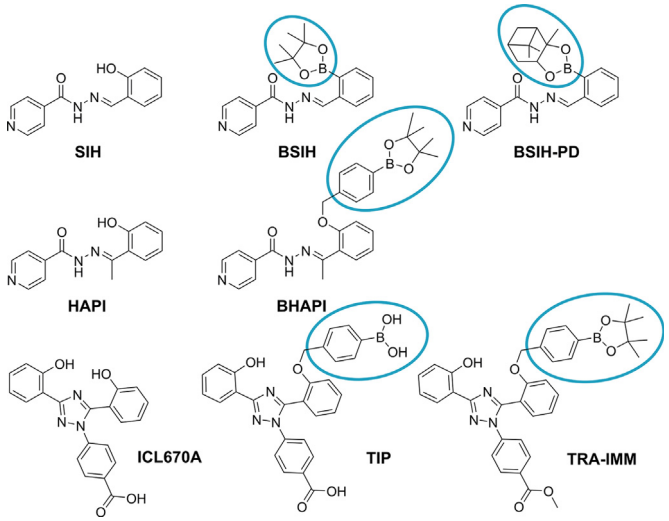


**Fig. 2.** Chemical structures of the compounds evaluated in this study. Chelators are shown on the left, and their corresponding oxidative stress-activated prochelators on the right, with masking group highlighted.

The first of the five prochelators examined in this study (Fig. 2), (*E*)-*N*′-[2-(4,4,5,5-tetramethyl[1,2,3]dioxaborolan-2-yl)-benzylidene] isonicotinohydrazide (BSIH), contains a boronic ester in place of a phenolic oxygen that is a key donor atom of the well-established experimental antioxidant aroylhydrazone metal chelator SIH [23–25]. (*E*)-*N*′-[2-(3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,2,3]dioxaborolan-2-yl)benzylidene] isonicotinohydrazide (BSIH-PD) is a BSIH analog that contains a pinanediol boronic ester instead of pinacol. The prochelator (*E*)-*N*′-[1-(2-((4-(4,4,5,5-tetramethyl-1,2,3-dioxoborolan-2-yl)benzyl)oxy)phenyl)ethylidene] isonicotinohydrazide (BHAPI) is derived from HAPI, an SIH analog with improved hydrolytic stability [26], but the direct boronic ester group of BSIH is replaced with a self-immolative spacer that undergoes spontaneous elimination after reacting with $H_2O_2$ [27]. In addition, two novel triazole prochelators were derived from the clinically used Fe chelator ICL670A, both with the self-immolative spacer and boronic acid (TIP; 4-[5-(2-((4-boronobenzyl)oxy)phenyl)-3-(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1-yl] benzoic acid) or its pinacol diol ester (TRA-IMM; methyl-4-[3-(2-hydroxyphenyl)-5-(2-((4-(4,4,5,5-tetramethyl-1,2,3-dioxoborolan-2-yl)benzyl)oxy)phenyl)-1*H*-1,2,4-triazol-1-yl] benzoate) [27]. In this study, the parent chelators and their prochelators were examined and compared for their relative affinity for Fe ions (before and after ROS exposure), ability to penetrate cells and bind labile cellular Fe, and potential to protect cardiac cells against the toxic action of model oxidative stress induced by cellular exposure to $H_2O_2$, as well as their inherent toxicity.

## Methods

### *Chemicals*

The tested compounds (Fig. 2), SIH, HAPI, BSIH, BHAPI, TRA-IMM, and TIP, were synthesized as described previously [23,25–29]. ICL670A was purified from a commercial pharmaceutical preparation from Novartis (Basel, Switzerland). BSIH-PD was synthesized at Duke University (Durham, NC, USA).

To prepare BSIH-PD, a portion of SIH-derived boronic acid (BASIH) was first prepared from equimolar quantities of isonicotinic acid hydrazide and (2-formylphenyl) boronic acid as previously reported [25]. Equimolar quantities of BASIH (0.20 g; 0.8 mmol) and (1*R*,2*R*,3*S*,5*R*)-pinanediol (0.136 g; 0.8 mmol) were added to 50 ml of a 9:1 mixture of toluene and dimethyl