Chelators to the Rescue: Different Horses for Different Courses!

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Chelators to the Rescue: Different Horses for Different Courses!

Cellular iron concentrations are tightly regulated by a complex network of signaling pathways that are triggered by high or low iron levels.[1,2] These pathways control the transport of iron in and out of the cell, as well as between different cellular compartments, and modulate the levels of the iron storage protein, ferritin.[1]

Although iron is an important element for life,[2−4] it can also be highly toxic to cells when accumulation occurs.[5,6] As such, a number of disease states are associated with deregulated iron homeostasis. These include disorders where tissue iron-loading occurs, including β-thalassemia and Friedreich's ataxia,[7−9] as well as iron-deficiency states such as anemia.[10] A therapeutic strategy for the treatment of iron-loading disorders is the use of iron chelators, which are agents that can enter cells and bind iron.[8,11] The siderophore, desferrioxamine (DFO), that is derived from the bacterium, *Streptomyces pilosus*, was one of the earliest chelators used in the clinics and is still implemented for the treatment of iron overload disease.[7,8,12] Recent studies have revealed that iron chelators may also be useful for the treatment of the mitochondrial iron-loading in Friedriech's ataxia, with agents such as pyridoxal isonicotinoyl hydrazone (PIH) showing benefits in reducing the cardiomyopathy associated with the disease.[13,14]

Interestingly, the potential of iron chelators extends further than just iron overload disorders (see Figure 1), with current research examining a number of different synthetic iron chelators for the treatment of cancer.[11,15,16] For example, the novel thiosemicarbazone iron chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT), is a promising therapeutic strategy for cancer treatment.[17] This is due to the ability of Dp44mT to bind iron and form redox-active complexes that produce reactive oxygen species (ROS), which are toxic to cancer cells.[18] The potential of iron chelators for the treatment of cancer is further highlighted by the entry of one such agent, Triapine, into multiple clinical trials.[16,19]

The many applications of chelators can partly be explained by the ability of structurally different ligands to form iron complexes that either promote or prevent ROS production in cells.[15,20,21] Hence, while the pro-oxidant nature of thiosemicarbazone chelators is useful for the design of highly cytotoxic agents for cancer treatment, the antioxidant property of desferrioxamine (DFO) is crucial for the treatment of iron-overload disease.[11]

In this issue of *Chemical Research in Toxicology*, Hruskova et al. extend the prospective use of iron chelators for the prevention of iron-mediated oxidative damage in the heart.[22] Such pathology can occur during myocardial ischemia-reperfusion injury, which is one of the primary causes of death following myocardial infarction.[23] Interestingly, the generation of ROS is well-known to occur during ischemia-reperfusion injury.[24] Hence, considering the involvement of iron in the production of damaging hydroxyl radicals, the use of iron chelators in patients following myocardial infarction may assist in preventing oxidative stress.[25] This is an important potential strategy for the treatment of cardiac injury since current efforts in the field largely focus on the use of ROS scavengers rather than the prevention of ROS production.

Previous *in vivo* studies using the iron chelator, DFO, showed that it was protective against posthypoxic-ischemic reperfusion injury in newborn lamb hearts.[26] In addition, hydroxyethyl starch-conjugated DFO was also shown to be effective in enhancing the recovery of regional myocardial function following coronary occlusion and reperfusion in dogs.[27] However, the efficacy of DFO was limited as it is highly hydrophilic, leading to low membrane permeability and poor absorption.[8] Thus, administration of DFO requires extensive periods of subcutaneous infusion causing poor patient compliance.[8] The generation of DFO coupled to hydroxyethyl starch can increase plasma concentrations of the drug, but unfortunately, it did not overcome the requirement for long infusions.[28] Hence, there was a need to improve the lipophilicity and membrane permeability of iron chelators.

As a strategy to increase membrane permeability and intracellular iron chelation, studies in the 1970's examined the iron chelator, pyridoxal isonicotinoyl hydrazone (PIH; Figure 2), which was synthesized by Schiff base condensation of pyridoxal with isonicotinic acid hydrazide.[29] PIH is orally active and was identified to be highly effective in permeating tissues to chelate iron.[29] The PIH analogue, salicylaldehyde isonicotinoyl hydrazone (SIH), was derived from its parent compound by replacing the pyridoxal moiety with the more hydrophobic salicylaldehyde group (Figure 2).[30] SIH was shown to readily enter cells to chelate the intracellular labile iron pool and thus efficiently prevented iron-mediated hydroxyl radical formation, which was in contrast to DFO.[30,31]

Indeed, SIH possessed considerable promise as this agent protects the heart against oxidative injury.[31−33] In cardiomyocyte cultures, SIH protected cells against hydrogen peroxide- or *tert*-butyl hydroperoxide-induced injury, while maintaining mitochondrial function and/or lysosomal integrity.[34,35] *In vivo*, SIH protected anthracycline-induced cardiotoxicity in rabbits, while showing good inherent tolerability with a low toxicity profile.[33] However, despite these promising results, the limitation of SIH was its relatively short biological half-life, as the ligand was prone to hydrolysis due to its labile hydrazone bond.[36] This limited the clinical potential of SIH because high or prolonged plasma concentrations of this agent were difficult to achieve and may be the reason for the reduction of its cardioprotective effects upon increasing its dose *in vivo*.[36]

Studies in the article by Hruskova et al. have overcome this limitation of SIH by increasing its plasma solubility through structural modifications that improve the stability of the ligand.[22] Such an advancement was achieved by replacing the aldimine hydrogen in SIH with a bulkier electron-donating alkyl group to decrease the nucleophilic attack of water on the hydrazone (C═N) bond (Figure 2). This approach generated a new series of ligands which were significantly more stable than SIH.[22] Importantly, these novel iron chelators maintained the ability

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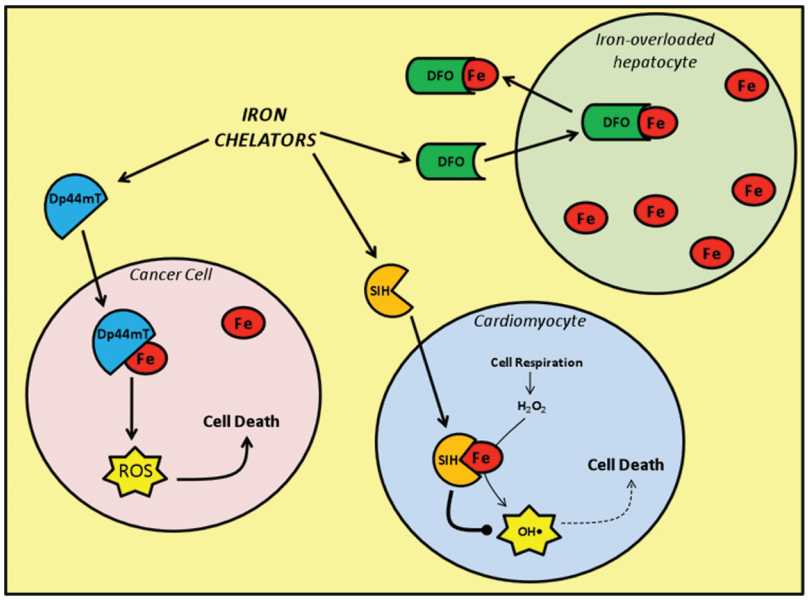

Figure 1. Iron chelators have numerous clinical uses that are determined by the molecular structure of these compounds. For instance, the natural siderophore, desferrioxamine (DFO), is primarily used for iron-overload diseases due to its ability to bind and remove iron (Fe) from iron-loaded hepatocytes. The thiosemicarbazone iron chelator, di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT), was designed for the treatment of cancer and is able to form a redox-active Fe complex in cancer cells, leading to the production of a toxic reactive oxygen species (ROS) that mediates cancer cell death. However, iron chelators such as salicylaldehyde isonicotinoyl hydrazone (SIH) function to reduce the formation of ROS by binding intracellular Fe and preventing it from participating in the Fenton reaction in ischemic myocardial cells. Hence, SIH may be useful for reducing ischemia-reperfusion myocardial injury following myocardial infarction.

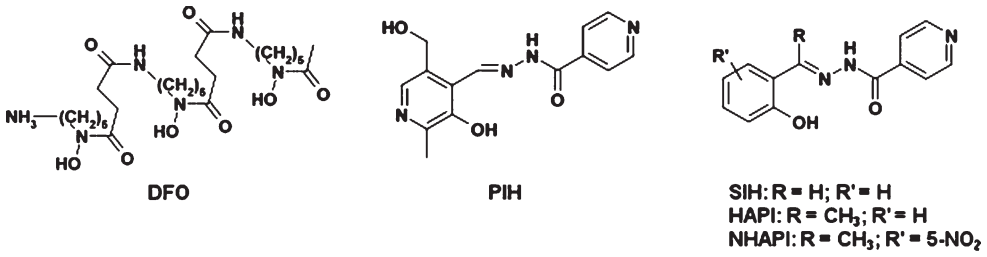

Figure 2. Chemical structures of DFO, pyridoxal isonicotinoyl hydrazone (PIH), SIH, and the new generation iron chelators, (*E*)-*N*′-[1-(2-hydroxyphenyl)ethyliden]isonicotinoylhydrazide (HAPI) and (E)-N′-[1-(2-hydroxy-5-nitrophenyl)ethyliden]isonicotinoylhydrazide (NHAPI).

to protect cardiomyocytes against iron-mediated oxidative injury and had lower toxicity than SIH in rat heart cells.[22] Among these new chelators is (*E*)-*N*′-[1-(2-hydroxyphenyl)ethyliden]isonicotinoylhydrazide (HAPI; Figure 2), which showed improved antioxidant and cytoprotective effects with little short-term toxicity when compared to those of SIH. These properties advocate the use of HAPI in acute situations such as ischemia-reperfusion injury. Another iron chelator, (*E*)-*N*′-[1-(2-hydroxy-5-nitrophenyl)ethyliden]isonicotinoylhydrazide (NHAPI; Figure 2), was identified as having the highest stability with negligible long-term toxicity and can potentially be useful for the treatment of atherosclerosis or heart failure.

The future development of these novel chelators will depend on investigation of their *in vivo* cardioprotective role and pharmacokinetic and toxicity profiles. These studies would include an examination of the protective role of these novel compounds in ischemia-reperfusion injury models (e.g., in rats and rabbits)[37,38] to assess their efficacy and plasma half-life. A comprehensive strategy to protect the intellectual property is also of importance to enable future commercial pharmaceutical development.

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REFERENCES

(1) Hentze, M. W., and Kuhn, L. C. (1996) Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proc. Natl. Acad. Sci. U.S.A. 93*, 8175–8182.

(2) Richardson, D. R., and Ponka, P. (1997) The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochim. Biophys. Acta 1331*, 1–40.

(3) Schejter, A., Ryan, M. D., Blizzard, E. R., Zhang, C., Margoliash, E., and Feinberg, B. A. (2006) The redox couple of the cytochrome c cyanide complex: The contribution of heme iron ligation to the structural stability, chemical reactivity, and physiological behavior of horse cytochrome c. *Protein Sci. 15*, 234–241.

(4) Atkin, C. L., Thelander, L., Reichard, P., and Lang, G. (1973) Iron and free radical in ribonucleotide reductase. Exchange of iron and Mossbauer spectroscopy of the protein B2 subunit of the *Escherichia coli* enzyme. *J. Biol. Chem. 248*, 7464–7472.

(5) Barnham, K. J., Masters, C. L., and Bush, A. I. (2004) Neurodegenerative diseases and oxidative stress. *Nat. Rev. Drug Discovery 3*, 205–214.

(6) Weinberg, E. D. (2010) The hazards of iron loading. *Metallomics 2*, 732–740.

(7) Wong, C., and Richardson, D. R. (2003) Beta-thalassaemia: emergence of new and improved iron chelators for treatment. *Int. J. Biochem. Cell Biol. 35*, 1144–1149.

(8) Olivieri, N. F., and Brittenham, G. M. (1997) Iron-chelating therapy and the treatment of thalassemia. *Blood 89*, 739–761.

(9) Napier, I., Ponka, P., and Richardson, D. R. (2005) Iron trafficking in the mitochondrion: novel pathways revealed by disease. *Blood 105*, 1867–1874.

(10) Fleming, M. D., Trenor, C. C., 3rd, Su, M. A., Foernzler, D., Beier, D. R., Dietrich, W. F., and Andrews, N. C. (1997) Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat. Genet. 16*, 383–386.

(11) Kalinowski, D. S., and Richardson, D. R. (2005) The evolution of iron chelators for the treatment of iron overload disease and cancer. *Pharmacol. Rev. 57*, 547–583.

(12) Chaston, T. B., and Richardson, D. R. (2003) Iron chelators for the treatment of iron overload disease: relationship between structure, redox activity, and toxicity. *Am. J. Hematol. 73*, 200–210.

(13) Whitnall, M., and Richardson, D. R. (2006) Iron: a new target for pharmacological intervention in neurodegenerative diseases. *Semin. Pediatr. Neurol. 13*, 186–197.

(14) Whitnall, M., Rahmanto, Y. S., Sutak, R., Xu, X., Becker, E. M., Mikhael, M. R., Ponka, P., and Richardson, D. R. (2008) The MCK mouse heart model of Friedreich's ataxia: Alterations in iron-regulated proteins and cardiac hypertrophy are limited by iron chelation. *Proc. Natl. Acad. Sci. U.S.A. 105*, 9757–9762.

(15) Buss, J. L., Greene, B. T., Turner, J., Torti, F. M., and Torti, S. V. (2004) Iron chelators in cancer chemotherapy. *Curr. Top. Med. Chem. 4*, 1623–1635.

(16) Wadler, S., Makower, D., Clairmont, C., Lambert, P., Fehn, K., and Sznol, M. (2004) Phase I and pharmacokinetic study of the ribonucleotide reductase inhibitor, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, administered by 96-h intravenous continuous infusion. *J. Clin. Oncol. 22*, 1553–1563.

(17) Whitnall, M., Howard, J., Ponka, P., and Richardson, D. R. (2006) A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. *Proc. Natl. Acad. Sci. U.S.A. 103*, 14901–14906.

(18) Chaston, T. B., Watts, R. N., Yuan, J., and Richardson, D. R. (2004) Potent antitumor activity of novel iron chelators derived from di-2-pyridylketone isonicotinoyl hydrazone involves fenton-derived free radical generation. *Clin. Cancer Res. 10*, 7365–7374.

(19) Kunos, C. A., Waggoner, S., von Gruenigen, V., Eldermire, E., Pink, J., Dowlati, A., and Kinsella, T. J. (2010) Phase I trial of pelvic radiation, weekly cisplatin, and 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, NSC #663249) for locally advanced cervical cancer. *Clin. Cancer Res. 16*, 1298–1306.

(20) Simunek, T., Sterba, M., Popelova, O., Adamcova, M., Hrdina, R., and Gersl, V. (2009) Anthracycline-induced cardiotoxicity: overview of studies examining the roles of oxidative stress and free cellular iron. *Pharmacol. Rep. 61*, 154–171.

(21) Rachmilewitz, E. A., Weizer-Stern, O., Adamsky, K., Amariglio, N., Rechavi, G., Breda, L., Rivella, S., and Cabantchik, Z. I. (2005) Role of iron in inducing oxidative stress in thalassemia: Can it be prevented by inhibition of absorption and by antioxidants? *Ann. N.Y. Acad. Sci. 1054*, 118–123.

(22) Hruskova, K., Kovarikova, P., Bendova, P., Haskova, P., Mackova, E., Stariat, J., Vavrova, A., Vavrova, K., and Simunek, T. (2011) Synthesis and initial in vitro evaluations of novel antioxidant aroylhydrazone iron chelators with increased stability against plasma hydrolysis. *Chem. Res. Toxicol.*10.1021/tx100359t.

(23) Turer, A. T., and Hill, J. A. (2010) Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. *Am. J. Cardiol. 106*, 1521–1522.

(24) DeBoer, D. A., and Clark, R. E. (1992) Iron chelation in myocardial preservation after ischemia-reperfusion injury: the importance of pretreatment and toxicity. *Ann. Thorac. Surg. 53*, 412–418.

(25) Horwitz, L. D., and Rosenthal, E. A. (1999) Iron-mediated cardiovascular injury. *Vasc. Med. 4*, 93–99.

(26) Shadid, M., Van Bel, F., Steendijk, P., Dorrepaal, C. A., Moison, R., Van Der Velde, E. T., and Baan, J. (1999) Effect of deferoxamine on post-hypoxic-ischemic reperfusion injury of the newborn lamb heart. *Biol. Neonate 75*, 239–249.

(27) Maruyama, M., Pieper, G. M., Kalyanaraman, B., Hallaway, P. E., Hedlund, B. E., and Gross, G. J. (1991) Effects of hydroxyethyl starch conjugated deferoxamine on myocardial functional recovery following coronary occlusion and reperfusion in dogs. *J. Cardiovasc. Pharmacol. 17*, 166–175.

(28) Dragsten, P. R., Hallaway, P. E., Hanson, G. J., Berger, A. E., Bernard, B., and Hedlund, B. E. (2000) First human studies with a high-molecular-weight iron chelator. *J. Lab. Clin. Med. 135*, 57–65.

(29) Ponka, P., Borova, J., Neuwirt, J., and Fuchs, O. (1979) Mobilization of iron from reticulocytes. Identification of pyridoxal isonicotinoyl hydrazone as a new iron chelating agent. *FEBS Lett. 97*, 317–321.

(30) Ponka, P., Richardson, D., Baker, E., Schulman, H. M., and Edward, J. T. (1988) Effect of pyridoxal isonicotinoyl hydrazone and other hydrazones on iron release from macrophages, reticulocytes and hepatocytes. *Biochim. Biophys. Acta 967*, 122–129.

(31) Bendova, P., Mackova, E., Haskova, P., Vavrova, A., Jirkovsky, E., Sterba, M., Popelova, O., Kalinowski, D. S., Kovarikova, P., Vavrova, K., Richardson, D. R., and Simunek, T. (2010) Comparison of clinically used and experimental iron chelators for protection against oxidative stress-induced cellular injury. *Chem. Res. Toxicol. 23*, 1105–1114.

(32) Simunek, T., Sterba, M., Popelova, O., Kaiserova, H., Adamcova, M., Hroch, M., Haskova, P., Ponka, P., and Gersl, V. (2008) Anthracycline toxicity to cardiomyocytes or cancer cells is differently affected by iron chelation with salicylaldehyde isonicotinoyl hydrazone. *Br. J. Pharmacol. 155*, 138–148.

(33) Sterba, M., Popelova, O., Simunek, T., Mazurova, Y., Potacova, A., Adamcova, M., Guncova, I., Kaiserova, H., Palicka, V., Ponka, P., and Gersl, V. (2007) Iron chelation-afforded cardioprotection against chronic anthracycline cardiotoxicity: a study of salicylaldehyde isonicotinoyl hydrazone (SIH). *Toxicology 235*, 150–166.

(34) Kurz, T., Gustafsson, B., and Brunk, U. T. (2006) Intralysosomal iron chelation protects against oxidative stress-induced cellular damage. *FEBS J. 273*, 3106–3117.

(35) Simunek, T., Boer, C., Bouwman, R. A., Vlasblom, R., Versteilen, A. M., Sterba, M., Gersl, V., Hrdina, R., Ponka, P., de Lange, J. J., Paulus, W. J., and Musters, R. J. (2005) SIH, a novel lipophilic iron chelator, protects H9c2 cardiomyoblasts from oxidative stress-induced mitochondrial injury and cell death. *J. Mol. Cell Cardiol. 39*, 345–354.

(36) Kovarikova, P., Klimes, J., Sterba, M., Popelova, O., Mokry, M., Gersl, V., and Ponka, P. (2005) Development of high-performance liquid chromatographic determination of salicylaldehyde isonicotinoyl hydrazone in rabbit plasma and application of this method to an in vivo study. *J. Sep. Sci. 28*, 1300–1306.

(37) Kim, Y. S., Kim, J. S., Kwon, J. S., Jeong, M. H., Cho, J. G., Park, J. C., Kang, J. C., and Ahn, Y. (2010) BAY 11-7082, a nuclear factor-kappaB inhibitor, reduces inflammation and apoptosis in a rat cardiac ischemia-reperfusion injury model. *Int. Heart J. 51*, 348–353.

(38) Black, S. C. (2000) In vivo models of myocardial ischemia and reperfusion injury: application to drug discovery and evaluation. *J. Pharmacol. Toxicol. Methods 43*, 153–167.