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RESEARCH ARTICLE

Evaluation of iron loading in four types of hepatopancreatic cells of the mangrove crab *Ucides cordatus* using ferrocene derivatives and iron supplements

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Abstract
The mangrove crab *Ucides cordatus* is a bioindicator of aquatic contamination. In this work, the iron availability and redox activity of saccharide-coated mineral iron supplements (for both human and veterinary use) and ferrocene derivatives in Saline *Ucides* Buffer (SUB) medium were assessed. The transport of these metallodrugs by four different hepatopancreatic cell types (embryonic (E), resorptive (R), fibrillar (F), and blister (B)) of *U. cordatus* were measured. Organic coated iron minerals (iron supplements) were stable against strong chelators (calcein and transferrin). Ascorbic acid efficiently mediated the release of iron only from ferrocene compounds, leading to redox-active species. Ferrous iron and iron supplements were efficient in loading iron to all hepatopancreatic cell types. In contrast, ferrocene derivatives were loaded only in F and B cell types. Acute exposition to the iron compounds resulted in cell viability of 70–95%, and to intracellular iron levels as high as 0.40 μmol L^{-1} depending upon the compound and the cell line. The easiness that iron from iron metallodrugs was loaded/transported into *U. cordatus* hepatopancreatic cells reinforces a cautionary approach to the widespread disposal and use of highly bioavailable iron species as far as the long-term environmental welfare is concerned.

Keywords Iron · *Ucides cordatus* · Fluorescence · Hepatopancreas · Mangrove crab

Introduction

Toxic metals such as cadmium, lead, and mercury are commonly considered environmental contaminants due to their persistence and low tolerance by organisms. Ions from transition metals such as copper, zinc, iron, manganese, or cobalt, on the other hand, are essential to the metabolism of most living systems, being important constituents of proteins and enzymes, with a plethora of biological roles (Sá and Zanotto 2013). However, an excess of these nutrients may be toxic, leading to undesirable reactions with unforeseen results such as free radical formation and oxidative damage. Therefore, a delicate balance must exist between deficiency and excess of these ions, by means of controlling their absorption and/or excretion (Halliwell and Gutteridge 1984).

Iron is a cofactor for a broad range of biochemical processes such as oxygen transport, cell respiration, citric acid cycle, lipid metabolism, gene regulation and DNA synthesis. In carrying these processes, iron is usually bound to the high-affinity heme group present in hemoglobin, myoglobin, and cytochromes (Andrews 2000). In many organisms, iron is necessary for oxygen transport, and in virtually all of them, the metal is at the basis of energy generation processes; therefore, there is a need to acquire iron from the environment. In recent years, iron transporter proteins have been identified in plasma from tarantula (*Pyura stolonifera*) and hemolymph from *Cancer magister* crabs (Huebers et al. 1982). Although it has not been fully recognized that these proteins are evolutionary precursors of vertebrate transferrin, they show similar properties (Huebers et al. 1982; Topham et al. 1988).

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Essential as it is, however, iron may engage into a series of redox reactions with oxygenated species within biological media, generating reactive oxygen species (ROS) through the Fenton and Haber–Weiss reactions, which will ultimately damage biomolecules, tissues, and organs (Meneghini 1997). Also, iron overload inside an organism may facilitate infection by competitive microorganisms, leading to sepsis (Muench 1989). Therefore, organisms should develop precise mechanisms to keep iron homeostasis (Cairo et al. 2006).

Emission of drugs is one of the major current environmental concerns. Drugs are by definition molecules designed to have strong biological effects at typically low concentrations. Among the main groups of drugs, antibiotics, hormones, and antidepressants should be especially harmful if released untreated into the environment (Bound et al. 2006), causing genotoxicity (Bound et al. 2006; Gil et al. 2007), and endocrine disruption (Hordern et al. 2009) in sensitive organisms. In this sense, iron metallodrugs such as iron supplements (both human and veterinary), sodium nitroprusside, or the proposed use of iron nanoparticles in diagnosis contain readily available forms of this metal, with possible deleterious ecotoxicological effects once emitted to the environment (Saravanan et al. 2015). Our previous findings showed that these compounds may be toxic to the aquatic organism *Artemia salina* (Vitorino et al. 2015).

The mangrove crab *Ucides cordatus* (Linnaeus 1763) is endemic to the coast of São Paulo State, Brazil, occurring both in ecological reserves and close to densely populated centers. It has been used as a bioindicator for both toxic (Pinheiro et al. 2013) and essential (Ahearn et al. 1999; Corrêa Junior et al. 2000; Chavez-Crooker et al. 2003a) metals. This animal is an interesting model for metal accumulation and aquatic toxicity (Ortega et al. 2014b). It has a relatively big and accessible organ, the hepatopancreas (HP). The hepatopancreas is considered as detoxification organ in crustaceans and reduce the adverse effects of toxic metals in the circulation (Ortega et al. 2011; Kang et al. 2012) and perform digestion and nutrient storage (Ortega et al. 2011). This organ is composed of four major cell types: E cells (embryonic cells originating other cell types), R cells (absorptive cells), F cells (fibrillar cells, secretion of digestive enzymes), and B cells (blister cells, intracellular digestion, and excretion of enzymes and xenobiotics) (Chavez-Crooker et al. 2001; Kang et al. 2012).

In this study, we compared two classes of iron compounds: the 3,5,5-trimethylhexanoyl (TMH) derivatives of ferrocene (Fc), TMH-Fc and $(TMH)_2$-Fc, and eight commercial human or veterinary iron supplements (composed of iron hydroxide cores with different organic coatings) in terms of iron availability, redox activity, and iron loading in *Ucides cordatus* HP cells in vitro. The iron availability and redox activity of iron compounds were studied in two relevant mimetic media, blood serum (HBS), a medium commonly used for application of metallodrugs, and hemolymph of crab *Ucides cordatus* (SUB), in order to compare the importance of salinity and pH in relation to the release of metal against iron chelators. In this way, it was possible to predict if these drugs, when discarded into the environment, have harmful effects on the hepatopancreatic cells studied and, ultimately, if they are a threat to the organisms that live in the mangrove. Here, we propose the determination of iron availability and redox activity of saccharide-coated mineral iron supplements and ferrocene derivatives. In addition, we propose an evaluation about the transport of these metallodrugs by hepatopancreatic cells of *Ucides cordatus* that could behave as aquatic contaminants.

Materials and methods

Iron supplements and Fc derivatives

Eight commercial iron supplements for either human or veterinary use with different coatings (dextran, glucoheptonate, glycinate, maltose) were procured from Brazilian sources. They are iron glucoheptonate (F1, Gleptoferril, Eurofarma), the dextran derivatives Biovet (F2, Biovet), Dexiron (F3, FATEC), iron dextran (F4, Uzinas Chimicas Brasileiras), Fertal (F5, Mogivet), Ferrodex (F6, Tortuga), and also iron glycinate (F7, Neutrofer, EMS Sigma Pharma) and iron maltose (F8, Noripurum, Nycomed). The Fc derivatives TMH-Fc and $(TMH)_2$-Fc were obtained by previously described methodologies (Nielsen and Heinrich 1993). Analysis of total iron concentration by means of atomic absorption spectroscopy was conducted using a Shimadzu AA-6300 equipment (Donadel et al. 2008).

Quantification of available iron by fluorescence quenching measurements

Stability of iron supplements and Fc derivatives was studied by means of a fluorescence quenching method with two iron-sensitive probes, calcein (Aldrich) and Fluorescent transferrin (Tf-Fl, obtained by a previously described methodology (Breuer and Cabantchik 2001), according to protocols already established by our group (Espósito et al. 2002). Physiological buffer HBS (Hepes Buffered Saline) was composed of hepes 20 mmol L^{-1}, NaCl 150 mmol L^{-1}, Chelex 1 g/100 mL; pH 7.4. Saline *Ucides* Buffer (SUB) was employed to mimic the hemolymph of *U. cordatus* (Ortega et al. 2011), consisting of NaCl 395 mmol L^{-1}; KCl 10 mmol L^{-1}; $NaHCO_3$ 2.5 mmol L^{-1}; NaH_2PO_4 2.5 mmol L^{-1}; Hepes 3.75 mmol L^{-1}; glucose 1 mmol L^{-1}; pH 7.8. In brief, 10 μL aliquots of iron compounds or 10 μL of iron standards (ferrous ammonium sulfate, FAS, in aqueous solution) with total Fe concentration of 0.0, 0.6, 1.2, 2.5, 5.0, 10.0, 20.0, and 40.0 μmol L^{-1} were transferred to flat and transparent 96-well microplates and treated with 190 μL of the fluorescent probe

(2 μmol L^{-1}) in the suited buffer. The mixture was allowed to react for 24 h at 22 ± 1 °C, then fluorescence endpoints were measured in a BMG FluoStar microplate reader ($\lambda_{exc}/\lambda_{em}$ = 485/520 nm). This analysis was carried out in eight repetitions (for every eight concentrations) and four replicates ($n = 4$) in different days.

Redox-active iron

A physiologically relevant model of the oxidative damage caused by excess iron involves its interaction with ascorbic acid in an aqueous saline buffer at 22 ± 1 °C (Esposito et al. 2003). The rate of reactive oxygen species formation in such setup is assessed fluorimetrically by the oxidation of the molecule dihydrorhodamine (DHR). High quantities of labile iron from a compound give rise to high oxidation rates; however, repetition of the experiment under the presence of a high-affinity iron chelator halts this oxidation and allows the quantification of redox-active iron (Esposito et al. 2003). In the flat bottom, transparent 96-well microplates were placed in 20 μL of the iron compound (40 μmol L^{-1}) or of Fe(NTA) standards, and 180 μL of a mixture of ascorbic acid and DHR (40 and 50 μmol L^{-1}, respectively) in either HBS or SUB buffer. The experiment was repeated with the addition of 100 μmol L^{-1} of deferiprone (Apotex, Canada), a strong iron chelator, to establish that oxidation was caused only by the presence of labile metal. Fluorescence was registered in a BMG FluoStar microplate reader $\lambda_{exc}/\lambda_{em}$ = 485/520 nm at 22 ± 1 °C for 1 h and the slopes of the kinetic curves were compared with the calibration curve in order to quantify the redox-active iron. This analysis was carried out in eight repetitions for 40 μmol L^{-1} and four replicates ($n = 4$) on different days.

Animals

Mangrove crabs *Ucides cordatus* (Decapoda, Ucididae) were collected at Pescadores beach, in São Paulo Coast (Lat. 24°10′ 59″S; Long. 46°47′20″W), the city of Itanhaém (Brazil), and transported to the Institute of Biosciences. Animals had an average weight of 150 g with 60 mm of carapace width. They were kept in filtered artificial seawater (20 ‰ salinity), gravel, filtered water, and pieces of brick to provide an emersion area for 7 days for acclimatization, during this period, animals were fed with white mangrove leaf; however, during the experimental period, the animals were not fed, according to Brazilian legislation and permission from IBAMA (Brazilian Institute of the Environment and Renewable Natural Resources). The photoperiod (12:12-h light:dark) was constant, as was the temperature (22 ± 3 °C). Only intermolt males were used to avoid different metabolic influences. For each set of experiments (two sets), five animals were used, then for the whole experiment, ten animals were used (Ortega et al. 2016).

Cell studies

Cells

The crabs were anesthetized at − 5 °C for 20 min and the HP was extracted. The hepatopancreatic cells were separated with 15 mL of extraction solution (NaCl 395 mmol L^{-1}; KCl 10 mmol L^{-1}; $NaHCO_3$ 2.5 mmol L^{-1}; NaH_2PO_4 2.5 mmol L^{-1}, Hepes 3.75 mmol L^{-1}; glucose 1 mmol L^{-1}, EDTA 0.9 mmol L^{-1}), which was stirred for 30 min at 200 rpm. The cell pellet was obtained by centrifugation at 1000 rpm for 10 min. The four cell types (E, R, F, and B) were obtained by means of a discontinuous sucrose gradient (10, 20, 30, and 40%, respectively) (Ortega et al. 2014a, b).

Cell viability

Aliquots of 180 μL of E, R, F, and B cell suspensions (23 × 10^4 cells/mL in SUB buffer) were transferred separately to 96-well microplates, then treated with 36 μL of iron compounds at 900 μmol L^{-1} or 50 ppm (final iron concentration). The microplate was incubated for 1 h at 25 °C, which is the time length of the transport test (see below). Quantification of live or dead cells was carried out by the Trypan blue (0.4%) method, after 1 h (Ortega et al. 2011).

Cell iron transport

Cells (23 × 10^4 cells/mL in SUB buffer) were treated with 1 μL of the cell-permeant iron probe acetometoxycalcein (CAL-AM) 5 mmol L^{-1} in dimethyl sulfoxide (Breuer et al. 1995) and 28 μL of 0.1 mmol L^{-1} probenecid (98 + %; Sigma). After, they were centrifuged for 1 h at 54×*g* and for 5 min at 405×*g* at 25 °C. The cell pellet was resuspended in EDTA-free extraction solution supplemented with probenecid at 0.1 mmol L^{-1} (Ortega et al. 2014a, b).

Aliquots of 180 μL of cell suspension were transferred to 96-well microplates and into a Biotek FLx800 Fluorescence Microplate Reader for fluorescence reading ($\lambda_{exc}/\lambda_{emis}$ = 485/520 nm). At $t = 30$ s, 36 μL of the different test compounds (5 mmol L^{-1}) were automatically injected into the solution containing the cells. Ferrous iron was used as a positive control. Full test time was 90 s. Intracellular iron quantification was calculated by means of a calibration curve of calcein fluorescence quenching by iron, in the same experimental setup. The experiment was carried out in three repetitions, with five replicates (Ortega et al. 2011; Sá and Zanotto 2013).

Before studying the iron compounds, a pilot test to assess the maximum and minimum of fluorescence intensity in cells was conducted. To this end, free Fe^{2+} (FAS) at 1 mmol L^{-1} was added to cells loaded with CAL-AM as described above. Good fluorescence quenching (~ 90%) was observed.

Statistical analysis

For the test performed, we used parametric analysis through analysis of variance (one-way ANOVA). For redox activity, viability, and for kinetic of intracellular iron, we used the Tukey post hoc test. The analysis was performed using Sigma Stat for Windows Ver 3.10, with a significance level of 0.05.

Results

The concentration of available iron was measured in two environmentally relevant mimics, HBS and SUB buffers, for human serum and HP, respectively. In both media, iron metallodrugs and ferrocene derivatives displayed no significant fluorescence quenching associated with iron in either calcein or Fl-Tf (data not shown). At higher total iron concentrations (20 to 80 μmol.L^{-1}), there was extensive interference of the color of the compounds (intense red).

There was no redox-active iron from iron supplements in any of the buffer systems employed. On the other hand, Fc and its derivatives displayed a considerable amount of labile iron (Table 1), which increased with buffer salinity. In the case of ferrocene, an increase in almost 4.6× (ANOVA, $p < 0.05$) was observed when the buffer was changed from HBS to SUB. For TMH-Fc and $(TMH)_2$-Fc, this activity increased around 4.2× and 10.5×, respectively (ANOVA, $p < 0.05$). Finally, for Fc, TMH-Fc, and $(TMH)_2$-Fc, there was an increase of redox activity after changing HBS to SUB buffer, with significative differences (ANOVA, $p < 0.05$), which correlated with the number of TMH substituents in the molecules.

Cell viability for the four types of HP cells (E, R, F, B) was high even under high (900 μmol L^{-1} or ~ 50 mg L^{-1}) iron concentrations. In comparison with the untreated ([Fe] = 0, no added iron) controls, Fc derivatives reached 90% of the viability on average, while iron supplements reached 70–95% (Fig. 1).

Iron supplements were absorbed by cell types, to a lower extent than Fc derivatives (Figs. 2, 3). Dextran or glucoheptonate iron was more available ($[Fe]_{int} \geq$ 0.3 μmol L^{-1}). There were no brand differences among the several iron dextran compounds (ANOVA, $p < 0.05$). Iron polymaltose (0.2 μmol L^{-1} < [Fe]int. < 0.3 μmol L^{-1}) and glycinate ([Fe]int. < 0.2 μmol L^{-1}) displayed lower iron loading (Table 2). Fc did not load iron in any cell type, and its derivatives were able to load iron in F and B cell types. Considering the cell types, Fe^{2+} was absorbed mainly by E cells (ca. 2.3 μmol L^{-1}), followed by R cells (ca. 1.8 μmol L^{-1}), F cells (ca. 1.7 μmol L^{-1}), and B cells (ca. 1.7 μmol L^{-1}) (ANOVA, $p < 0.05$) (Table 2).

Discussion

Fluorimetric detection of iron allows for the high throughput screening of (i) stability of iron compounds against dissociation, (ii) iron loading to cells (Breuer et al. 1995), and (iii) anti- or pro-oxidant activity of selected iron compounds (Esposito 2003). In the absence of mobilizing agents, all iron metallodrugs were found to be stable against high-affinity metal chelators such as calcein and transferrin, both in SUB and in HBS buffer, releasing virtually no detectable iron to the probes (data not shown). This is in line with our previous findings referring to the low iron availability from iron hydroxides coated with sugars (Vitorino et al. 2015). However, in the presence of ascorbic acid, a major constituent of biological fluids and cells (Naidu 2003), Fc derivatives did generate free, redox-active iron species (Table 1). Concentrations were higher for SUB buffer probably because its higher chloride content (three times higher than

Table 1 Redox-active iron from different compounds (40 μmol L^{-1} in iron, total concentration) in HBS and SUB buffers with ascorbate acid 40 μmol L^{-1} and DHR 40 μmol L^{-1} at 22 ± 1 °C for 40 min (average ± SD; $n = 4$)

Iron compound	HBS		SUB	
	Redox-active iron (μmol L^{-1})	Redox-active iron (%)	Redox-active iron (μmol L^{-1})	Redox-active iron (%)
Iron metallodrugs (all)	nd	nd	nd	nd
Fc	$1.20 \pm 0.17^{(a)}$	3.0 ± 0.4	$5.53 \pm 0.38^{(c)}$	13.8 ± 0.9
TMH-Fc	$3.80 \pm 0.17^{(b)}$	9.5 ± 0.4	$16.10 \pm 0.97^{(d)}$	40.3 ± 2.4
TMH_2-Fc	$3.26 \pm 0.15^{(b)}$	8.2 ± 0.4	$34.16 \pm 0.74^{(e)}$	85.4 ± 1.8

Different letters indicate significant differences after ANOVA ($p < 0.05$). In the HBS medium, there is a significant difference between Fc and ferrocene derivatives. In SUB medium, everyone is different from each other. Among HBS and SUB media, there is also a significant difference between Fc and ferrocene derivatives

nd not detected

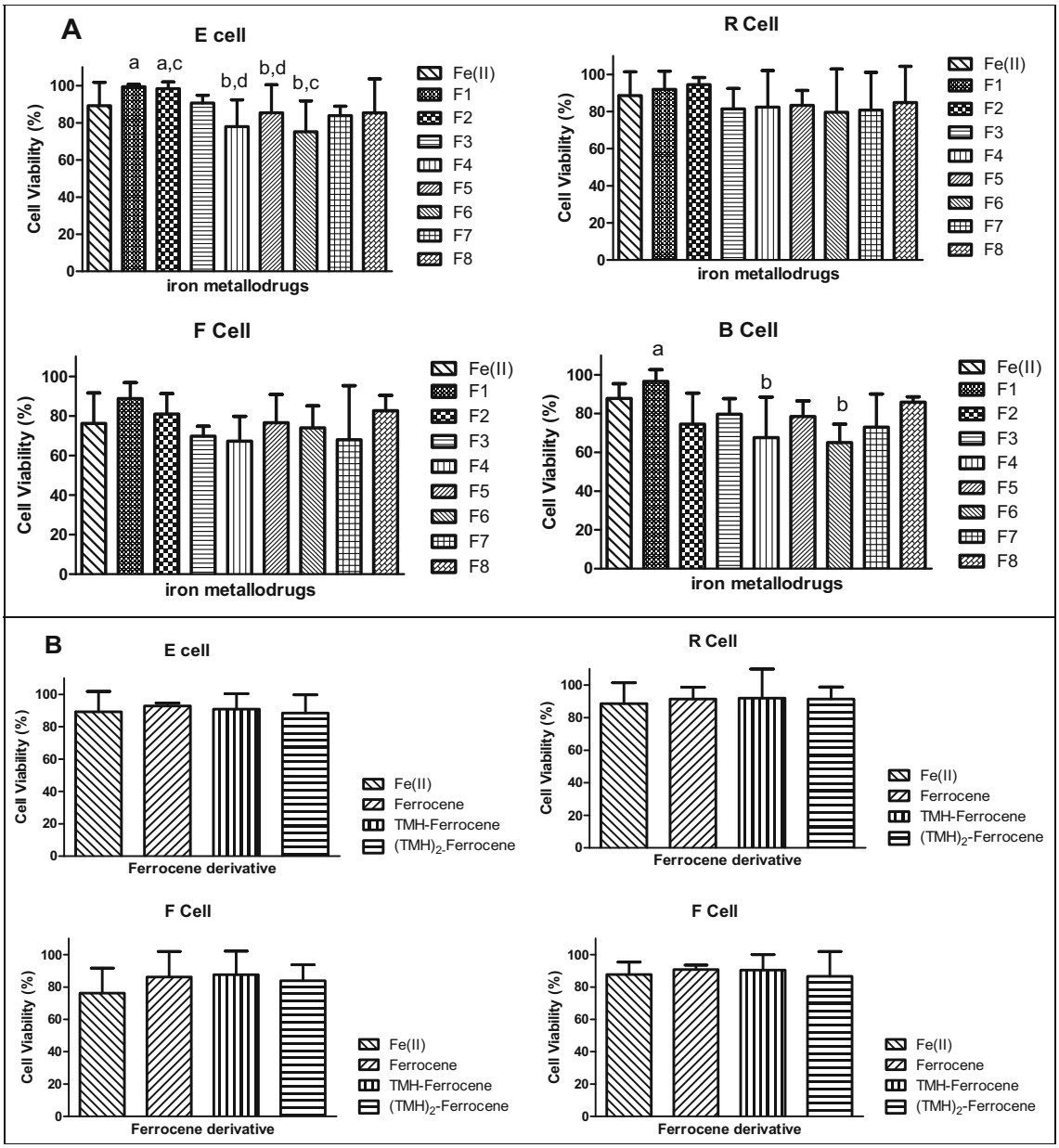

Fig. 1 Viability (average ± SD; $n = 5$) of cell types (E, R, F, and B) of the mangrove crab *Ucides cordatus* after 1 h exposure to **a** iron metallodrugs: glucoheptonate (F1, Gleptoferril), the dextran derivatives Biovet (F2, Biovet), Dexiron (F3), iron dextran (F4), Fertal (F5), Ferrodex (F6), and also iron glycinate (F7, Neutrofer) and iron maltose (F8); **b** ferrocene derivatives. Total iron concentration was 900 μmol L^{-1}. Different capital letters represent statistical differences ($P < 0.05$)

in HBS) assists the solubilization of the reduced iron species. Coated compounds such as the iron supplements were stable against ascorbate reduction, which was also observed in artificial seawater (Vitorino et al. 2015). Therefore, the initial speciation of iron will determine its ability to engage in biotic or abiotic redox reactions promoted by endogenous cofactors such as ascorbate.

HP cells are classified according to the function they develop and their stage of differentiation (Ortega et al. 2014b). Embryonic (E) cells are the non-differentiated precursors of the other cell types, with high energy demand to afford their intensive mitosis. They further differentiate into resorptive (R) or fibrillar (F) cells; the first type displays nutrient storage roles (lipids, glycogen, and metal ions) and the second develops vacuoles for storage of toxic metals more frequently than the other cell types (Ortega et al. 2011). The F cells further differentiate into blister or vesicular (B) cells, which possess a characteristic large, single vacuole designed for metabolism of xenobiotics, wrapped by a thin cell membrane (Hirsch and Buchmann 1930). A fifth cell type can also be found in crustaceans, mainly Malacostraca, such as *Penaeus*

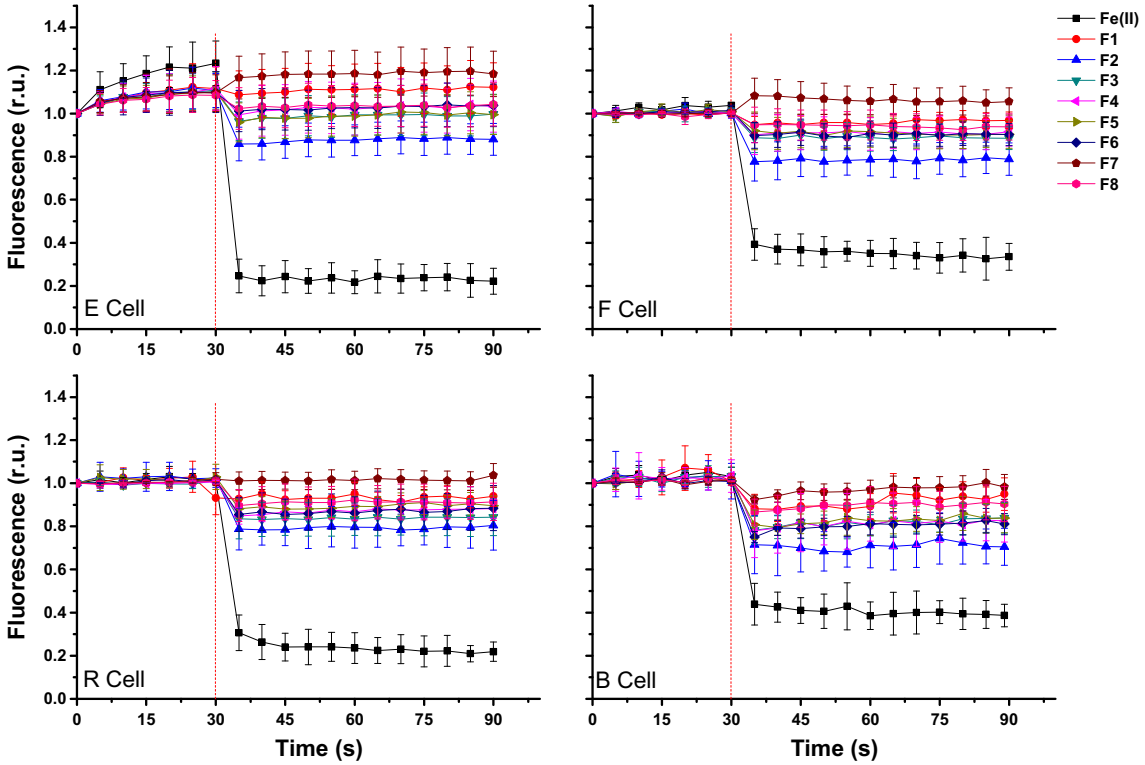

Fig. 2 Kinetics of intracellular iron accumulation in HP cells from *U. cordatus* (average ± SD; $n = 5$) for different cell types, after treatment with iron supplements (900 μmol L^{-1}) at $t = 30$ s. r.u. = relative fluorescence units. Embryonic cells (E), resorptive cells (R), fibrillar cells (F), and blister cells (B) are represented. Iron supplements are Gleptoferril (F1, iron glucoheptonate), the dextran derivatives Biovet (F2), Dexiron (F3), Ferro dextrano (F4), Fertal (F5), and Ferrodex (F6), as well as Neutrofer (F7, iron glycinate) and Noripurum (F8, iron maltose)

semisulcatus, being called M (midgut) cells; however, this other cell type is not present in *Ucides cordatus* (Marcolin et al. 2008).

Cell viability assays (Fig. 1) indicate that acute exposure to all studied compounds does not induce significant toxic effects. Iron supplements and ferrocene derivatives are usually well tolerated by human cell lines such as HepG2 and THP-1 macrophages (Liu 2011; Praschberger et al. 2015). Similar results showed high viability in the presence of cadmium (Ahearn et al. 2004; Ortega et al. 2016), copper, calcium, and zinc (Chavez-Crooker et al. 2003b; Ahearn et al. 2004) ions, and suggest that the detoxifying properties of the HP organ are related to tolerance in its four cell types (Ahearn et al. 2004).

Cell viability results show that the generation of redox-active species is not directly related to acute toxicity. Since iron is an essential trace element, this is an expected outcome since organisms usually have a high buffer capacity to handle excess iron. However, if the iron compounds significantly alter the intracellular iron levels in *U. cordatus*, this could lead to long-term noxious effects either for the individuals (e.g., Genotoxicity (Gagné 2014)) or the environment (e.g., eutrophication leading to unsustainable population levels).

The kinetic profiles of iron transport by the different cells treated with the iron compounds are shown in Figs. 2, 3, as fluorescence quenching signals (the higher the amount of iron released into the cell, the lower the fluorescence). After signal stabilization, it was possible to quantify the amount of intracellular iron by means of a calibration curve with calcein (Table 2). Different cell types responded differently to the iron compounds.

Possible mechanisms postulated for the internalization of these materials were endocytosis, with iron release promoted at acidified endosomes (Doherty and McMahon 2009), or membrane reduction of Fe^{3+} by ferrireductases (Viarengo and Nott 1993; Ahearn et al. 1999) followed by transport by divalent ion channels (Chavez-Crooker et al. 2003b; Pinto et al. 2014). In contrast to iron supplements (Fig. 2), only the TMH derivatives of Fc displayed some iron release (Fig. 3) in F or B cell types. The hexanoyl substitution makes the Fc derivatives more lipophilic and able to diffuse through cell membranes, as previously shown in other biological models as *Artemia salina* (Vitorino et al. 2015). Cells with digestive and

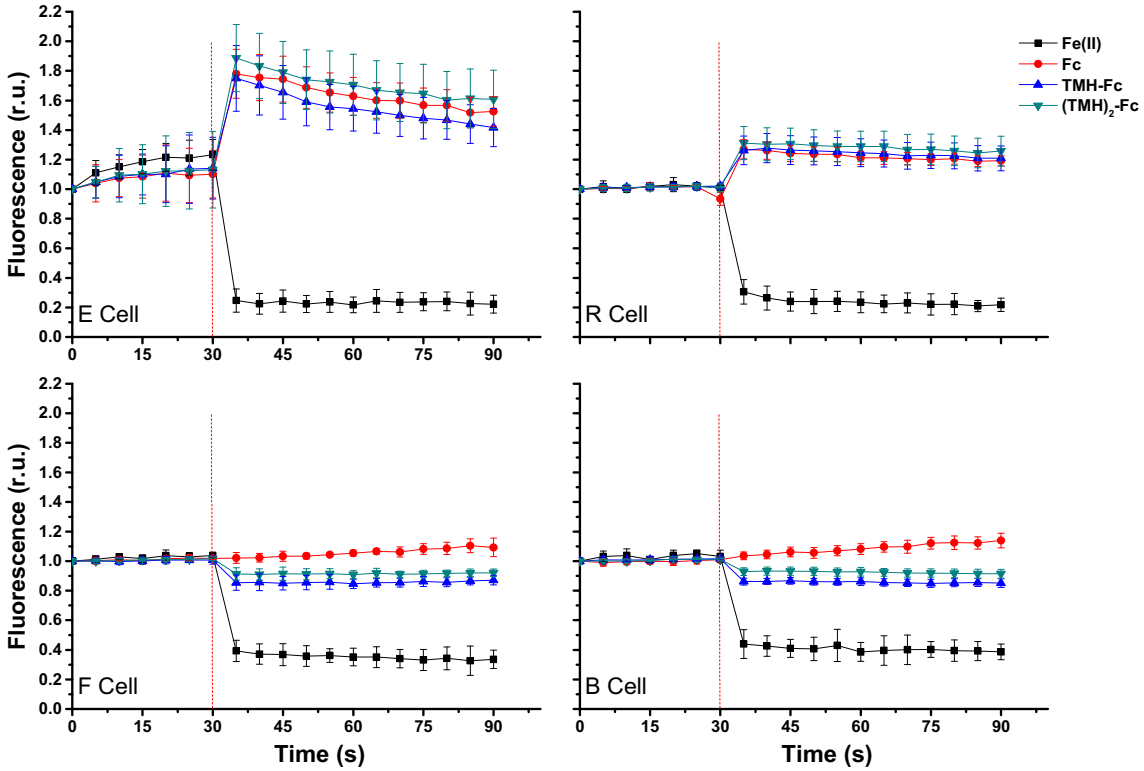

Fig. 3 Kinetics of intracellular iron accumulation in HP cells (average ± SD; $n = 5$) from *U. cordatus* for different cell types, after treatment with ferrocene derivatives (900 μmol L^{-1}) at $t = 30$ s. r.u. = relative fluorescence units. Embryonic cells (E), resorptive cells (R), fibrilar cells (F), and blister cells (B) are represented

detoxifying roles (F and B) typically absorbed iron for all instances. In E and R cell of hepatopancreas, Fc derivatives were the only ones that showed no transport of iron. Interestingly, for this class of compounds, even the high metabolism displayed by E cells was not enough to transport iron, in opposition to what was observed for iron supplements, where the saccharide coating is precisely designed to enhance cell transport. For any given compound, and when all cell types displayed iron transport, E cells were good or the best accumulators, reflecting

Table 2 Intracellular iron concentrations (μmol L^{-1}) in HP (hepatopancreas) cells of *Ucides cordatus* at 22 ± 3 °C (average ± SD; $n = 5$ independent experiments).

Iron compound	E*	R	F	B
Fe(II)	2.31 ± 0.08	1.80 ± 0.08	1.73 ± 0.16	1.72 ± 0.03
F1: glucoheptonate[(a)]	0.36 ± 0.04	0.24 ± 0.06	0.32 ± 0.05	0.32 ± 0.06
F2: dextran[(a)]	0.33 ± 0.08	0.27 ± 0.07	0.31 ± 0.06	0.34 ± 0.06
F3: dextran[(a)]	0.34 ± 0.05	0.30 ± 0.08	0.32 ± 0.06	0.31 ± 0.05
F4: dextran[(a)]	0.33 ± 0.09	0.29 ± 0.07	0.27 ± 0.07	0.34 ± 0.02
F5: dextran[(a)]	0.30 ± 0.08	0.28 ± 0.05	0.31 ± 0.06	0.31 ± 0.06
F6: dextran[(a)]	0.33 ± 0.07	0.24 ± 0.09	0.30 ± 0.05	0.33 ± 0.06
F7: glycinate[(b)]	0.15 ± 0.04	0.13 ± 0.06	0.15 ± 0.06	0.19 ± 0.04
F8: polymaltose[(c)]	0.27 ± 0.06	0.17 ± 0.08	0.25 ± 0.05	0.21 ± 0.09
Fe(II)	2.29 ± 0.03	1.82 ± 0.11	1.72 ± 0.08	1.69 ± 0.05
Fc	nd	nd	nd	nd
TMH-Fc	nd	nd	0.26 ± 0.02	0.36 ± 0.08
$(TMH)_2$-Fc	nd	nd	0.20 ± 0.03	0.31 ± 0.04

Asterisk means that E cells present significant differences in relation to all cell types and compounds studied, after ANOVA, $p < 0.05$. Different small letters indicate significant differences after ANOVA ($p < 0.05$), in relation to groups of compounds

nd not detectable, *E* embryonic cells, *R* resorptive cells, *F* fibrillar cells, *B* blister cells

their demand for nutrients to allow for high cell division and differentiation.

Conclusions

Iron availability and redox activity of iron metallodrugs, both commercial and candidate, were determined against calcein and fluorescent transferrin by quenching fluorescence and by oxidation of dihydrorhodamine, respectively. Free, redox-active iron was only detected upon mediation by ascorbate. Iron supplements and ferrocene derivatives did not induce acute toxicity; however, increased iron loading was observed especially for E cells, and for coated supplements or lipophilic Fc derivatives. Therefore, the long-term effects of increased metal load caused by bioactive iron species should be considered.

Conflict of interest The authors declare that they have no conflicts of interest.

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