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New 8-hydroxyquinoline and catecholate iron chelators: Influence of their partition coefficient on their biological activity

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Abstract

Four new hexadendate chelators, three hydroxyquinoline-based, Csox, O-Trensox, Cox750, and one catecholate-based CacCam—which have comparable skeletal structures and pFe, but widely different partition coefficients, (Kpart), 0.01, 0.02, 1 and 3.2 respectively, have been tested for their iron chelating efficacy *in vitro* by two methods. First, by their ability to remove iron from ferritin in solution or second, to remove iron from iron-loaded hepatocytes *in vitro*. Our objective was to ascertain the importance of Kpart and pFe, on the biological efficiency of the molecule. Previous studies proposed that an ideal value of Kpart of 1 should give maximum biological activity. Mobilization of iron by Csox and CacCAM from ferritin was similar and furthermore more efficient than desferrioxamine B. In the iron-loaded hepatocyte cultures, the three hydroxyquinoline chelators, although showing diversity in terms of lipophilicity, appeared to be very similar in their capacity to chelate iron. CacCAM, the unique catecholate, was the most efficient of the molecules tested, as well as being the least toxic in the cellular model despite having the lowest value of pFe. In conclusion, the use of the partition coefficient and pFe, as tools for predicting biological activity of iron chelators should be not generalized. Further studies are required in order to understand the influence of the structure on the biological activity of the molecule. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: 8-hydroxyquinoline; O-Trensox; Partition coefficient; Hepatocyte cultures; Iron-dextran

1. Introduction

No mechanisms exist for the excretion of iron from man such that it essential that iron intake is strictly controlled. Therefore patients with chronic hemolytic anemias who receive blood transfusions and/or iron supplementation to correct their apparent iron deficiency may ultimately develop tissue iron overload, which can cause extensive toxicity. Unless such individuals receive chelation therapy death may occur during the third decade of life from chronic tissue malfunction due to the excessive storage of iron in many tissues, particularly the heart [1]. Iron overload, which is evident in thalassemia syndromes, presents a major health problem in many developing countries and it is estimated that there are 60,000 thalassemia major births annually [2].

Only the natural siderophore chelator DFO has been approved for therapeutic use to remove the excessive accumulation of tissue iron. However, it has one major drawback in that it is necessary to administer subcutaneously over long time periods such that it poses some difficulties with compliance as well as the high cost of patient care [3,4].

Therefore, there is an urgent need for iron chelators that show oral efficacy for the treatment of iron overload. For the development of such molecules, attempts have been made to correlate the chemical structure as well as certain physical parameters with their biological activity. In addition, the resulting iron complex formed should be non-

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Abbreviations: Kpart, partition coeffient; DFO, desferrioxamine B; TRX, O-Trensox; pFe = $-\log[Fe^{3+}_{aq}]$ at pH 7.4 with [Ligand] = 10 μM and [Fe] = 1 μM; LDH, lactate dehydrogenase; and PBS, phosphate-buffered saline.

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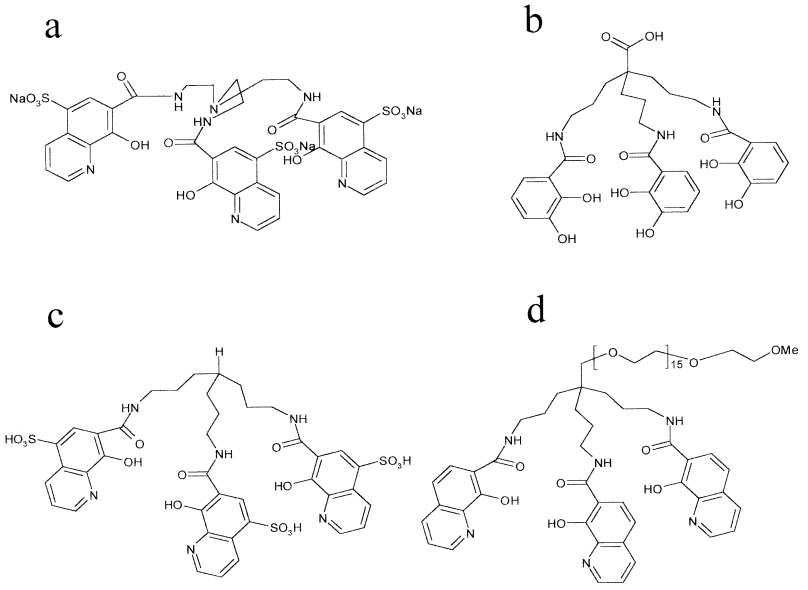

Fig. 1. Diagrammatic representation of the chelators: a) TRX; b) CacCAM; c) Csox; and d) Cox750.

toxic and the coordinated iron should be protected from interaction with either hydrogen peroxide or oxygen. Four main families have been studied so far: catecholate [5], hydroxamate [6], carboxylate [7], and hydroxypyridone ligands [8].

Many investigators have suggested that lipid solubility is an important factor for chelator efficacy, its partition coefficient, Kpart, should not be greater than 1 to allow cellular transit both of the free ligand into the cell and of the iron complex out of the cell without causing cellular damage. Kpart can be assessed by the distribution of the chelator between an aqueous solution and an organic solvent, e.g. water and octanol. The ability of the chelator to cross the membrane is clearly an important factor in its efficacy [11], which may be attributable to its lipophilicity [13], (which would have a decisive effect upon biliary excretion) and hydrophilicity [9]. It was confirmed [12] that a Kpart close to unity appeared to be optimal for the pyridinone-facilitated removal of iron from hepatocytes. However certain molecules show efficacy despite a low Kpart. For example, DFO has a low partition coefficient, the entry of labelled desferrioxamine B and its subsequent release within hepatocytes, is essentially a controlled diffusion process [10], although Porter suggested that there might be some facilitated uptake [11]. Salicylaldehyde isonicotinoyl hydrazone, which was the fastest acting of all tested chelators, also showed a relatively low lipophilicity of both the free ligand (Kpart = 0.35) and the iron complex (Kpart = 0.1). More recently, Lowther *et al.* [14] have compared a new hydroxipyridinone (CGP 65015) with L1, which has a higher affinity for iron and a higher Kpart. Despite these qualities, *in vitro* and *in vivo* studies predict that absorption of CGP 65015 in man is likely to be incomplete because hydrogen-bound acidity of the compound might be expected to limit its membrane permeability. Such results would indicate that membrane permeability is not a limiting factor for good iron chelators as stated in our previous studies [10].

Other physical parameters which may play a crucial role in the design of clinically useful oral chelators are molecular weight and pFe [15]. However even if a chelator possesses a high pFe value this does not necessarily imply suitability for the clinical situation. In 1998, Lowther *et al.* [16] presented a monoethyl ester of HBED (*N*,*N*′-bis(2hydroxybenzyl)ethyldiamine-*N*,*N*′-diacetic acid) (CGP 75254A). Importantly, its affinity for iron (log KML = 30.7), although several log units lower than that of the parent compound HBED, is as high or even higher than that of most orally active iron chelator from other chemical classes. Several investigators have shown that, to cross a lipid bilayer, the compound needs to proceed through a desolvation step in order to accommodate the nonpolar environment of the inner layer of the phospholipid membranes [17]. The efficacy of CGP 75254A could be partly explained by its high solubility in cyclohexane, an aprotic solvent.

Careful examination of the existing literature has shown that many parameters have been used to predict the chelating ability of the compounds: lipophilicity, hydrogen bounds, molecular weight, pFe, solubility/desolvation. Therefore, in this present study four new chelators (Fig. 1) have been investigated *in vitro*, three of which have a comparable structure but differ with respect to both Kpart and pFe (Table 1). The comparison of their efficacy to remove iron *in vitro* both from ferritin in solution and iron-loaded hepatocyte cultures has facilitated our knowledge in the importance of Kpart and pFe on the biological efficacy of the molecule to chelate iron.

Table 1
Partition coefficient (P)* and pFe

Ligand	P free ligand	pFe
DFO	0.03	26.9
Csox	0.01	29.9
O-Trensox	0.02	29.5
Cox750	1	29.5
Caccam	3.2	27.5

2. Materials and methods

2.1. Materials

Horse spleen ferritin and DFO was from Sigma. TRX, Cox750, CacCAM, and Csox was supplied by P. Baret (CNRS UMR 5616; Laboratoire d'Etudes Dynamiques et Structurales de la Sélectivité, Université Joseph Fourier, Grenoble, France). Iron-Dextran was from Vifor Pharmaceutical.

For the preparation of ^{55}Fe-dextran solution, a solution of $^{55}FeCl_3$ was prepared in 1 mL of 0.1 N hydrochloric acid to which sucrose, 100 mg, was added and dissolved with stirring with a glass rod. Sodium hydroxide, 100 μL, 10 N, was then added with stirring (solution 1).

Iron-dextran 5%, 2 mL, corresponding to 100 mg of Fe, was placed in a 10-mL vial. Sodium hydroxide, 1 N, was added with stirring with a magnetic stirrer until the pH of the solution was greater than 11 (solution 2).

Solution 1 is added to solution 2 with stirring and the pH of this solution is adjusted to 5.7, to obtain the ^{55}Fe-dextran solution.

2.2. Iron mobilization from ferritin

Ferritin with an iron concentration of 300 μM (corresponding to a protein concentration of 10^{-7} M) was incubated with the chelator under investigation, at a concentration of 1 mM, in 0.2 M MOPS buffer, pH 7.4, and then incubated at 37° for 6 h. Iron release was measured at regular intervals by using λmax of 428, 519, and 585 nm and e (L/mol·cm) of 2800, 5000, and 5000 for DFO, CacCAM, and Csox, respectively. Results are presented as means ± SD for five samples at each time point.

2.3. Hepatocyte isolation

Sterile procedures were utilized for this procedure. Glassware was sterilized by autoclaving and media filtration through 0.22-μm Millipore filters. The liver collagenase perfusion method [18–19] was used as the basis for the preparation of hepatocytes. Male rats (Wistar, 150–200 g) were anesthesised by an intraperitoneal injection of Nembutal, sodium pentabarbitone, 0.6 mg/kg. The portal vein and vena cava were cannulated with 18-gauge and 16-gauge Teflon catheter (Baxter) respectively. The liver was initially perfused with 100 mL of PBS containing 0.5 mM EGTA and then for 15–25 min with recirculating PBS containing 60 mg of collagenase Type IV and 5 mM $CaCl_2$. The perfusate was equilibrated with O_2 and maintained at 37°C during the procedure. After perfusion, the liver was removed and scraped into 50 mL of PBS. The resulting suspension of hepatic cells was filtered sequentially through 30-, 60-, and 100-μm polyester meshes. The cell suspension obtained after liver perfusion was centrifugated for 2 min at 60 g in a swinging bucket rotor. The pellet contained the hepatocytes. The hepatocytes were then suspended in 100 mL of culture medium and counted in a Bürker cell. Cellular viability, as judged by trypan blue exclusion was greater than 90%. Two millilitres of the cellular suspension at a density of 0.75×10^6 cells/mL was incubated in Dulbecco's modified Eagle's medium containing 10% (v/v) foetal calf serum, 4 mM glutamine, 20 mM glucose, and transferred to polystyrene culture dishes (Nunc), which had been precoated with collagen. To obtain the collagen coating culture dishes were incubated for at least 5 hr at 37°C with 2 mL of purified bovine dermal collagen (Cellon-Luxembourg), which was diluted in PBS at a concentration of 30 μg/mL.

The hepatocytes were cultivated at 37° in a water-saturated atmosphere under CO_2/air (7:93 v/v). After 4-hr incubation, almost all of the cells had adhered to the collagen membrane and the culture medium was replaced by Williams E medium (Life Technologies) to which insulin (10 nM), linoleic acid complexed to albumin, dexamethasone (10 μg/mL), ascorbic acid (0.1 mM), and epidermal growth factor (50 μg/mL) had been added. This is known as hormone-defined media and is without foetal calf serum. The cultures were maintained: a) in control condition (with neither iron nor chelators present); b) in the presence of iron alone; or c) in the presence of iron plus chelators. Chelators were added on day 2 (i.e. 48 hr after culture establishment).

2.4. ^{55}Fe release from hepatocyte cultures

For the mobilization experiments following iron-dextran loading, hepatocyte cultures were incubated in Williams E medium on a coating of 60 μg of rat collagen. Hepatocytes were iron-loaded by using ^{55}Fe-dextran at a concentration of 1.5 mM for 48 hr. After iron loading, the hepatocytes were incubated with the chelators at a concentration of 50 μM, and ^{55}Fe iron release was measured over a period of 10 days.

2.5. LDH assay

LDH activity were measured in the culture medium as an index of cytotoxicity, employing LDH/LDL Sigma Diagnostic kit. The results were expressed in terms of LDH release into the medium per μg of cellular protein.

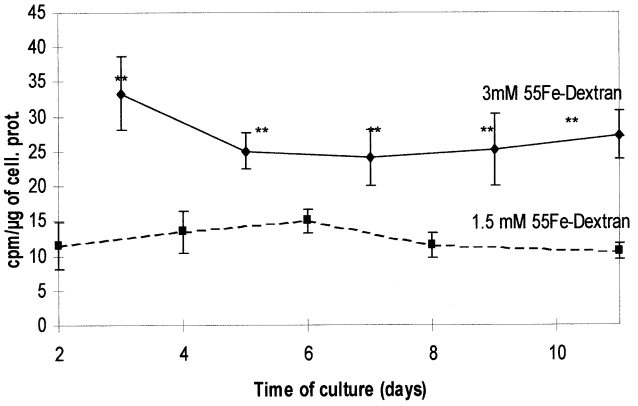

Fig. 2. Stability of ^{55}Fe within hepatocytes for 11 days after incubation of isolated hepatocytes with either 1.5 and 3 mM ^{55}Fe-dextran. Results are expressed as cpm/μg. Bars indicate means ± SD. (*) and (**) Statistically significant differences compared with corresponding 1.5 mM ^{55}Fe-dextran group ($P < 0.05$ and $P < 0.01$, respectively).

2.6. Antiproliferative effect of chelators

Fao cells derived from the hepatic cell line of rat Reuber [20] were maintained by subculture in a culture medium (50% Ham F12 medium, 50% NCTC 135 medium) supplemented with 10% foetal calf serum, 2 mM glutamine, 7×10^{-7} M hemisuccinate hydrocortisone and containing per mL: streptomycin (50 μg), penicillin (7.5 IU), bovine insulin (5 μg), bovine serum albumin (1 mg), $NaHCO_3$ (2.2 mg). Antiproliferative effects of chelators were evaluated in the cell line by measuring ^{3}H-methyl-thymidine incorporation (1 μCi/mL of medium) in the DNA of exposed cells for 48 hr at 20 or 50 μM chelator. DNA synthesis was evaluated by measuring [^{3}H]methyl-thymidine incorporation into trichloroacetic acid-precipitated DNA. Results were expressed as a percentage of control values.

2.7. Protein assay

To determine the total protein per well, the media were decanted and the cell cultures extensively rinsed with PBS. Cells were sonicated for 15 sec. The protein content was determined according to the method of Lowry *et al.* [21] by using bovine serum albumin as standard in the range of 1–20 μg.

3. Results

3.1 Iron mobilization from ferritin

Mobilization of iron from ferritin at pH 7.4 was significantly faster and more efficacious with Csox and CacCAM than with DFO (Fig. 2). These two chelators mobilize approximately 2-fold more iron atoms per molecule of ferritin than DFO after 6 hr of incubation.

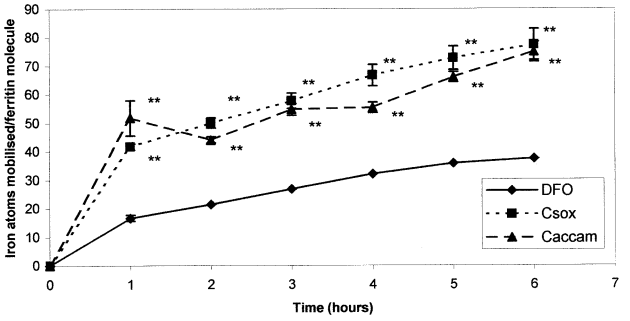

Fig. 3. Mobilization of iron by 1 mM DFO, Csox, or Caccam from ferritin at pH 7.4. Results are means ± SD for five samples at each time point. (*) and (**) Statistically significant differences compared with corresponding DFO group ($P < 0.05$ and $P < 0.01$, respectively).

3.2. Stability of iron within the hepatocytes

Figure 3 shows that there was little alteration in the radioactive iron content of the hepatocytes over a 11-day period, the amount of radioactive iron incorporated into the hepatocyte being proportional to the iron content of the media.

3.3. ^{55}Fe release from hepatocytes cultures

The mobilization of iron by DFO, Csox, CacCAM, Trensox, and Cox750, at a chelator concentration of 50 μM, from rat hepatocyte cultures, which had been loaded *in vitro* with ^{55}Fe-dextran, is presented in Fig. 4a and b. After 10 days, DFO had mobilized 70% of the iron, whereas Trensox and Cox750 exhibited efficiency of ~34% (Fig. 4a). Csox is the more efficient hydroxy-quinoline ligand with a mobilization of 45% after 10 days of culture (Fig. 4b). CacCAM the catecholate compound shows an efficiency comparable to DFO with 60% of iron mobilized after 10 days of culture (Fig. 4b).

3.4. LDH assay

Chelators with hydroxy-quinoline groups (Trensox, Cox750, and Csox) induced a small release of LDH into the medium between the 4th and 6th day of culture (Fig. 5a and b). DFO showed either no toxicity (Fig. 5a) or a small release on day 6 and 8 of the culture (Fig. 5b). CacCAM is not toxic (Fig. 5b). The hepatocytes, which were loaded with iron-dextran *in vitro*, did not show any toxicity, as reflected by the low release of LDH into the culture media (Fig. 5b).

3.5. Antiproliferative effect of chelators

In order to determine whether the chelators affects Fao cell proliferation, we measured [^{3}H]methyl-thymidine incorporation into these cells after addition of each chelator at a concentration of either 20 or 50 μM after 48 hr. Figure 6a

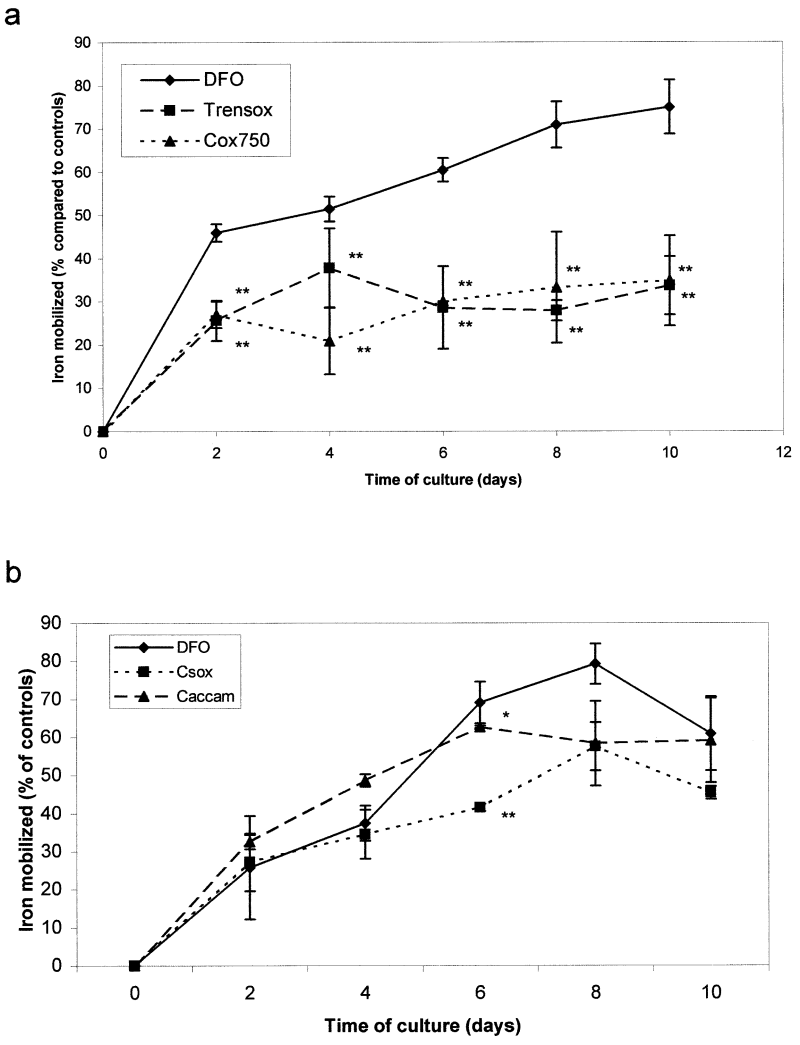

Fig. 4. a) Mobilization of iron from ^{55}Fe-dextran preloaded rat hepatocyte cultures by 50 μM DFO, TRX, or Cox 750. Results are means ± SD for three samples at each time point; b) Mobilization of iron from ^{55}Fe-dextran preloaded rat hepatocyte cultures by 50 μM DFO, Csox or Caccam. Results are means ± SD for three samples at each time point. (*) and (**) Statistically significant differences compared with corresponding DFO group ($P < 0.05$ and $P < 0.01$, respectively).

shows a significant decrease in DNA synthesis in the TRX, and Cox750-treated cells at a concentration of 20 μM. DFO did not induce a diminution of DNA synthesis. Exposure of the cultures to 50 μM chelators confirmed that these last results were dose dependent (Fig. 6b).

4. Discussion

Hepatocytes are crucially important, not only in general intermediary metabolism, but also in iron metabolism. Iron uptake from non-transferrin sources may be an important factor in determining the extent of parenchymal iron loading that may be either safely sequestered into ferritin and hemosiderin, or cause toxicity, manifested by the consequences of oxidative stress within cells provoked by Fenton chemistry. Therefore, there is clearly a need for a hepatocyte, *in vitro* system, which will allow the investigation of the efficacy of new classes of iron chelators prior to their utilization in animal models. Previous studies which used hepatocytes for the evaluation of iron chelators, introduced labeled iron into these cells via transferrin [22–24], or alternatively, loaded the cells *in vivo* with iron, using iron dextran [25] carbonyl iron [26], or ferrocene [27]. In these present studies we have used ^{55}Fe dextran *in vitro* to load hepatocytes, which was shown to be stable with time and did not exhibit any toxicity (Figs. 2 and 5a and b).

Iron mobilization from ferritin by Csox and CacCAM seems to be very effective; however, CacCAM was not more effective than Csox. Desferrioxamine mobilizes only 37 iron atoms per molecule of ferritin after 6 hr of incubation, which is at variance to a previous study where 160 iron atoms per molecule of ferritin were mobilized [28]. This could be explained by the different protocol used to purify

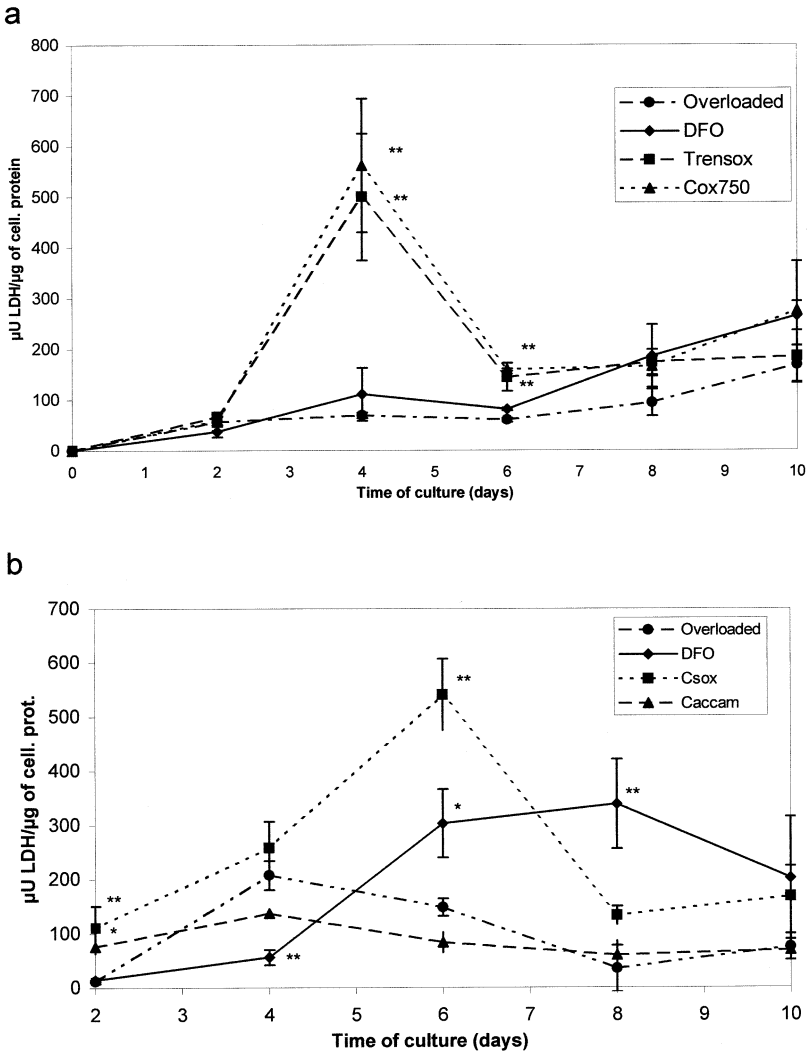

Fig. 5. a) Release of LDH in rat hepatocyte cultures maintained for 10 days after iron loading, in the presence of DFO (50 μM), TRX (50 μM), and Cox750 (50 μM). Results are means ± SD for three samples at each time point. b) Release of LDH in rat hepatocyte cultures maintained for 10 days after iron loading, in the presence of DFO (50 μM), Csox (50 μM), or Caccam (50 μM). Results are means ± SD for three samples at each time point. (*) and (**) Statistically significant differences compared with corresponding overloaded group ($P < 0.05$ and $P < 0.01$, respectively).

the ferritin. Indeed, unpublished results showed that ferritin prepared by thermal degradation of the contaminating proteins release its iron more easily that the one prepared by acidic degradation.

As shown in the study of Rakba *et al.* [28], iron mobilization from hepatocytes by TRX is significantly less efficient than DFO (Fig. 4a). Cox750, with the same pFe as TRX but with a partition coefficient closer to one (Table 1) had nevertheless the same efficiency (Fig. 4a). This is contrary to the work of Porter *et al.* [11], which predict that a better chelating efficacy could be obtained for molecules with a partition coefficient of 1. Csox, TRX, and Cox750 had a diminished chelating efficacy by comparison to DFO (Fig. 4b). Although each of these molecules shows diversity in term of lipophilicity, they appear very similar in their capacity to remove iron from a cellular model. As already concluded by Thomas *et al.* [29], lipophilic solubility does not seem to be a necessary prerequisite for transmembrane iron transport.

Of all of the molecules investigated in this present study CacCAM, was the more efficient despite the fact that it had the lowest value of pFe. Interestingly, although this molecule had the highest lipophilicity, it was the least toxic molecule (Fig. 5b). During the first 6 days of the culture it even appeared to be protective, the release of LDH was significantly lower than the iron-loaded group (*t*-test, $P < 0.05$). The 8-hydroxyquinoline compounds were toxic between the 4th and 6th day of culture, which confirms previous studies [30–32].

In 2000, Rakba *et al.* [33] suggested that TRX and DFO act on cell cycle progression via distinct mechanisms and/or chelate intracellular metal pools with different efficiency. Inhibition of DNA synthesis was evident is for both TRX and Cox750 but not for DFO. Therefore two 8-hy-

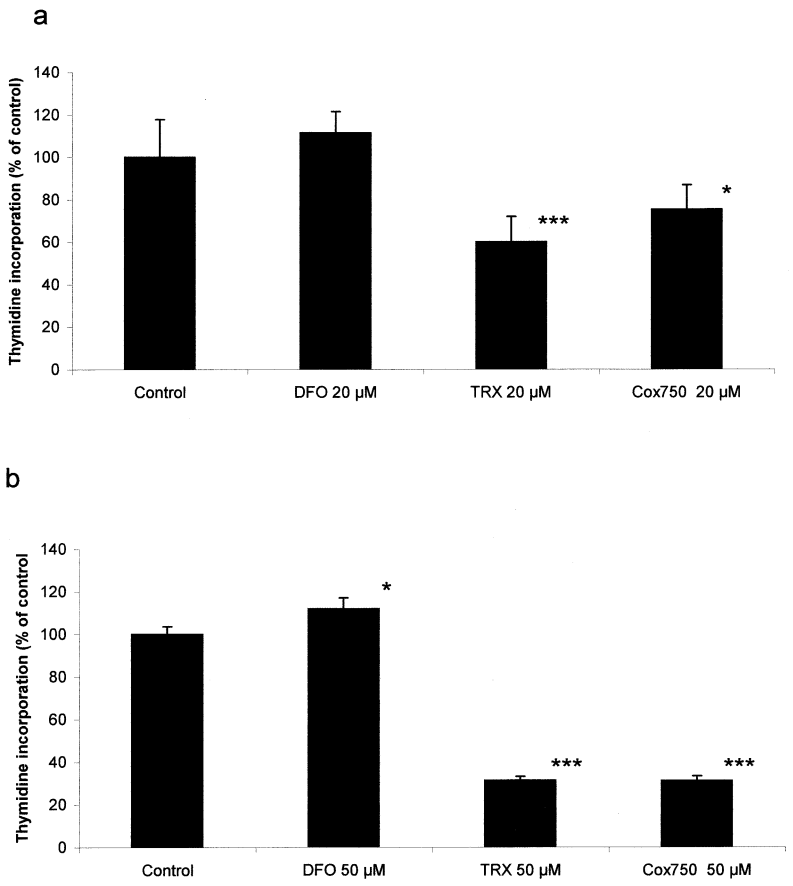

Fig. 6. a) [^{3}H]methylthymidine incorporation into DNA in Fao cells derived from the hepatic cell line of rat Reuber cultures maintained for 48 hr under control conditions and in the presence of 20 μM chelators; b) [3H]methylthymidine incorporation into DNA in Fao cells derived form the hepatic cell line of rat Reuber cultures maintained for 48 hr under control conditions and in the presence of 50 μM chelators. (*) and (***) Statistically significant differences compared with corresponding control group ($P < 0.05$ and $P < 0.001$ respectively).

droxyquinoline, which show different lipophilicity, have similar characteristics with respect to their effect on cellular proliferation.

Due to its good chelator efficacy in both the non-cellular and cellular models combined with its low toxicity, Cac-CAM seems to be a promising iron chelator. Further *in vivo* studies are now required to ascertain whether similar efficacy is found. *In vitro*, the 8-hydroxyquinoline molecules (TRX, Csox, and Cox750) are less efficient than DFO in removing iron from the iron-loaded hepatocytes. Furthermore, they show toxicity when added to hepatocyte cultures. The differences in hydrophobicity/hydrophilicity of the molecules did not yield the expected results. Indeed, the most hydrophilic molecule had the same activity as the most hydrophobic molecule. Further studies are required for a better understanding of the influence of chemical structure on the biological activity of the molecules to chelate iron.

References

[1] Bergeron RJ, Wollenberg M, Wiegan J. An investigation of desferrithiocin metabolism. J Med Chem 1994;37:2889–95.
[2] Cao A. Results of programs for antenatal detection of thalassemia in reducing the incidence of the disorder. Blood Rev 1987;1:169–76.
[3] Hershko C, Pinson A, Link G. Iron chelation. Blood Rev 1990;4:1–8.
[4] Brittenham GM. Development of iron chelating agents for clinical use. Blood 1992;80:569–74.
[5] Rodgers SJ, Lee CH, Ng CY, Raymond KN. Ferric iron sequestering agents. Inorg Chem 1987;26:1622–5.
[6] Bergeron RJ, Wiegand J, McManis JS, Perumal PT. Synthesis and biological evaluation of hydroxamate-based iron chelators. J Med Chem 1991;34:3182–7.
[7] Martell AE, Motekaitis RJ, Clarke ET, Harrison JJ. Synthesis of N,N′-di(2-hydroxybenzyl)ethylenediamine-N,N′-diacetic acid derivatives. Can J Chem 1986;64:449–56.
[8] Hider RC, Kontoghiorghes G, Silver J. UK Patent GB2118176, 1982.
[9] Meyer–Brunot HG, Keberle H. Biliary excretion of ferrioxamines of varying liposolubility in perfused rat liver. Am J Physiol 1968;214:1193–200.
[10] Laub R, Schneider YJ, Octave JN, Trouet, A, Crichton RR. Cellular pharmacology of deferrioxamine B and derivatives in cultured rat hepatocytes in relation to iron mobilization. Biochem Pharmacol 1985;34:1175–83.
[11] Porter JB, Gyparaki M, Burke LC, Huehns ER, Sarpong P, Saez V, Hider RC. Iron mobilization from hepatocyte monolayer cultures by chelators: the importance of membrane permeability and the iron-binding constant. Blood 1988;72:1497–503.
[12] Dobbin PS, Hider RC, Hall AD, Taylor PD, Sarpong P, Porter JB, Xiao G, van der Helm D. Synthesis, physicochemical properties, and

biological evaluation of N-substituted 2-alkyl-3hydroxy-4(1H)-pyridinones: orally active iron chelators with clinical potential. J Med Chem 1993;36:2448–58.
[13] Zanninelli G, Glickstein H, Breuer W, Milgram P, Brissot P, Hider RC, Konijn AM, Libman J, Shanzer A, Cabantchik IZ. Chelation and mobilization of cellular iron by different classes of chelators. Mol Pharmacol 1997;51:842–52.
[14] Lowther N, Fox R, Faller B, Nick H, Jin Y, Sergejew T, Hirschberg Y, Oberle R, Donnely H. In vitro and in situ permeability of a "second generation" Hydroxypyridinone oral iron chelator: correlation with physico-chemical properties and oral activity. Pharm Res 1999;16: 434–40.
[15] Hider RC, Khodr H, Liu ZD, Tilbrook G, Optimisation of pFe values of iron(III) ligands with clinical potential. Metal Ions Biol Med 1998;5:51–5.
[16] Lowther N, Tomlinson B, Fox R, Faller B, Sergejew T, Donnely H. Caco-2 cell permeability of a new (hydroxybenzyl)ethylenediamine oral iron chelator: correlation with physicochemical properties, and oral activity. J Pharm Sci 1998;87:1041–5.
[17] Faller B, Spanka C, Sergejew T, Tschinke V. Improving the oral bioavailability of the iron chelator HBED by breaking the symmetry of the intramolecular H-Bond Network. J Med Chem 2000;43:1467–75.
[18] Berry MN, Friend DS. High yield preparation of isolated rat liver parenchymal cells. J Cell Biol 1969;43:506–20.
[19] Jeejeebhoy KN, Ho J, Greenberg GR, Phillips MJ, Robertson BA, Sodtke U. Albumin, fibrinogen, and transferrin synthesis in isolated rat hepatocyte suspensions. Biochem J 1975;146:141–55.
[20] Deschatrette J, Weiss MC. Characterization of differentiated and dedifferentiated clones from a rat hepatoma. Biochimie 1974;56: 1603–11.
[21] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin reagent. J Biol Chem 1951;193:265–75.
[22] Baker E, Vitolo ML, Webb JM. Iron chelation by pyridoxal isonicotinyl hydrazone and analogues in hepatocytes in culture. Biochem Pharmacol 1985;34:3011–7.
[23] Baker E, Page MP, Torrance J, Grady RW. Effect of desferrioxamine, rhodoturolic acid and cholyl hydroxamic acid on transferrin and iron exchange with hepatocytes in culture. Clin Physiol Biochem 1985;3: 277–88.
[24] Baker E, Wong A, Peter H, Jacobs A. Desferrithiocin is an effective iron chelator in vitro and in vivo but ferrithiocin is toxic. Br J Haematol 1992;81:424–31.
[25] Tyson CA, LeValley SE, Chan R, Hobbs PD, Dawson MI. Biological evaluation of some ionophore-polymeric chelator combinations for reducing iron overload. J Pharmacol Exp Ther 1984;228:676–81.
[26] Sharma BK, Bacon BR, Britton RS, Park CH, Magiera CJ, O'Neill RO, Dalton N, Smanik P, Speroff T. Prevention of hepatocyte injury in lipid peroxidation by iron chelators and α-tocopherol in isolated iron loaded rat hepatocytes. Hepatology 1990;12:31–9.
[27] Ward RJ, Florence AL, Baldwin D, Abiaka C, Roland F, Ramsey MH, Dickson DPE, Peters TJ, Crichton RR. An animal model of primary haemochromatosis. Eur J Biochem 1991;202:405–10.
[28] Rakba N, Aouad F, Henry C, Caris C, Morel I, Baret P, Pierre JL, Brissot P, Ward RJ, Lescoat G, Crichton RR. Iron mobilisation and cellular protection by a new synthetic chelator O-Trensox. Biochem Pharmacol 1998;55:1797–806.
[29] Thomas F, Baret P, Imbert D, Pierre JL, Serratrice G. Partition coefficients (free ligands and their iron(III) complexes) and lipophilic behavior of new abiotic chelators. Correlation to biological activity. Bioorg Med Chem Lett 1999;9:3035–40.
[30] Shen AY, Chen CP, Roffler S. A chelating agent possessing cytotoxicity, and antimicrobial activity: 7-morpholinomethyl-8-hydroxyquinoline. Life Sci 1999;64:813–825.
[31] Leanderson P, Tagesson C. Iron bound to the lipophilic iron chelator, 8-hydroxyquinoline, causes DNA strand breakage in cultured lung cells. Carcinogenesis 1996;17:545–50.
[32] Forsbeck K, Nilsson K, Kontoghiorghes GJ. Variation of iron accumulation, transferrin membrane binding and DNA synthesis in the K-562 and U-937 cell lines induced by chelators and their iron complexes. Eur J Haematol 1987;39:318–25.
[33] Rakba N, Loyer P, Gilot D, Delcros JG, Glaise D, Baret P, Pierre JL, Brissot P, Lescoat G. Antiproliferative and apoptotic effects of O-Trensox, a new synthetic iron chelator, on differentiated human hepatoma cell lines. Carcinogenesis 2000;21:943–51.