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Recent advances in cancer treatment by Iron Chelators

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Abstract

The development of new therapeutic alternatives for cancers is a major public health priority. Among the more promising approaches, the iron depletion strategy based on metal chelation in the tumoral environment has been particularly studied in recent decades. After a short description of the importance of iron for cancer cell proliferation, we will review the different iron chelators developed as potential chemotherapeutics. Finally, the recent efforts to vectorize the chelating agents specifically in the microtumoral environment will be discussed in detail.

Graphical abstract
Despite the continuing progress in terms of treatment, screening and care, cancer remains a major public health problem, mainly due to intra-tumoral heterogeneity and chemotherapeutic resistance. In addition to conventional treatments, the efforts of the scientific community are focused on the search for new therapeutic targets and greater selectivity with the ultimate aim of a personalized treatment. Among the therapeutic alternatives developed, certain nutrients involved in many metabolic processes are being studied as potential targets of anticancer treatments. A better understanding of biochemical mechanisms has shown that the transition metals, like iron, copper and zinc, are essential for cell growth. Due to their intensive proliferation, tumor cells require large quantities of this type of nutrient, particularly iron, and the disruption of iron metabolism within cancerous cells (increased uptake and restricted elimination) contributes to their proliferation. In vitro studies have shown that iron chelators, initially designed for the treatment of iron overload, present very interesting antiproliferative properties. By trapping (mobilizing) the iron within tumors, these molecules offer new perspectives for anticancer treatments. Thus, the chelation of metals seems a promising alternative in the development of new original therapeutic agents.

Iron - indispensable for life. Iron is a trace element involved in numerous metabolic processes and is thus indispensable for life. At the biological level, it is found in two oxidation states, ferrous iron (Fe²⁺) and ferric iron (Fe³⁺). Its ability to pass from one state to another explains why it is an essential constituent of many metalloproteins that exploit this oxidation-reduction property. Almost all organisms require iron as a micronutrient as it is needed for the biosynthesis of macromolecules, cell division and proliferation and is involved in a wide variety of cellular processes such as the production of energy, the transport of oxygen and the synthesis of DNA. For example, ribonucleotide reductase (RR), a key enzyme in DNA synthesis that catalyzes the transformation of ribonucleotides into deoxyribonucleotides, has two atoms of iron in its active site.

In humans, the amount of iron is maintained at a constant level, estimated at around four grams. In the organism, iron is found in a heme form (as a porphyrin complex) within hemo-proteins, which represents about 80% of iron, and in a non-heme form distributed between storage (ferritin and hemosiderin) and transport proteins (transferrin). Iron homeostasis is a finely regulated process and its deregulation leads to either deficiency or overload, which is damaging for the organism due to the production of reactive oxygen species (ROS). The formation of ROS, and particularly of very reactive hydroxyl radicals via the Fenton reaction, provokes mutations and serious cellular damage. As a result of these reactions, a high intracellular level of iron has been identified as a risk factor for the development of cancers.

The perfect balance: metabolism and regulation of iron. The intestinal absorption of iron occurs essentially in the duodenum where non-heme iron from food, in the ferric Fe³⁺ form, is reduced to ferrous Fe²⁺ iron by the ferrireductase Duodenal cytochrome b (Dcytb). Fe²⁺ is then internalized at the apical membrane of enterocytes by the Divalent Metal Transporter 1 (DMT1) and becomes part of the Labile Iron Pool (LIP) (Figure 1).

Little is known about the mechanism of absorption of heme iron but it represents only a small part of ingested iron. The heme is internalized by a specific membrane transporter, the Heme-Carryer Protein 1 (HCPI), then, once in the endoplasmic reticulum, the iron is liberated by the Heme Oxygenase 1 (HO-1) and joins the LIP (Figure 1).

The ferrous iron in the enterocyte is liberated into the blood circulation by a transmembrane protein, the Ferropotin 1 (Fpn1), oxidized into ferric iron by a ferroxidase, the hephaestin, then transported into the plasma linked to a protein, the transferrin.

From the LIP, the iron may be either used directly (protein synthesis, DNA synthesis, mitochondrial synthesis), or exported as previously by Fpn1, or stored. Ferritin is the main storage agent of iron; it is a heteropolymer made up of 24 subunits that can accommodate up to 4500 iron atoms in ferric Fe³⁺ form in its center. This store protects the organism against the oxidizing and toxic effects of free iron. The organism can mobilize these reserves if the need for iron increases or the input is insufficient.

Deregulation of the iron balance and cancer. As mentioned above, in physiological conditions the level of iron is controlled by various hepatic, enterocyte and macrophagic mechanisms, which maintain an ideal balance. The deregulation of this subtle homeostasis of iron is one of many malfunctions that characterize cancerous cells. Although iron can induce the formation of ROS, it is not itself considered carcinogenic, but rather it acts as a cofactor that favors tumor growth. Much research has shown the disruption of iron
homeostasis in cancerous cells, associated with the deregulation of many other genes. This leads to phenotypic changes that give these cells a survival advantage by stimulating their proliferation.

In most cancerous cells, the need for a large increase in iron is linked to an acceleration of cell division. In tumor cells, the rise in DNA synthesis explains the increased activity and expression of ribonucleotide reductase (RR). This results in the increased expression of TR1 leading to more iron entering the cell (Figure 2, cancerous cell). Moreover, the levels of Fpn1, the expression of ferritin and of the protein STEAP3 are disrupted in cancerous cells. Thus, in summary, a rise in the entry of iron, a fall in its elimination and a disruption of its storage all result in an accumulation of iron. This becomes available for DNA synthesis and thus cell proliferation, or for the formation of ROS responsible for cellular damage.

Iron chelators as chemotherapeutic agents. Chelators were initially developed for the treatment of iron overload in pathologies such as beta thalassemia, before it was shown that they led not only to iron depletion but also to a reduction in tumor growth. This inhibition of cell proliferation occurs by different processes that regulate the cell cycle, angiogenesis or the suppression of metastases. In fact, iron chelators have many molecular targets resulting in their impact in terms of mechanisms of action. This explains why many iron chelators have been developed for anticancer therapy in both in vitro and in vivo studies in the last twenty years.

When designing chelators for clinical applications, their selectivity towards iron as well as the stability of the complexes formed in the physiological environment are both crucial factors. To be entirely satisfactory, the coordination of iron requires six donor atoms in an octahedral configuration with the metallic iron in the middle. When a ligand contains two donor atoms, it is called bidentate. Tridentate ligands have three donor atoms and hexadentate ligands have six atoms (Figure 3).

Desferrioxamine (DFO, Figure 4), a siderophore used historically in the clinical treatment of iron overload, was the first iron chelator to be examined for its anticancer properties. Many studies have shown its antiproliferative activity against a wide variety of tumor cell lines. For example, DFO inhibits, in vitro and in vivo, the growth of melanoma and hepatoma cells by blocking their proliferation in phase S of the cell cycle. During preliminary clinical trials, this chelator also proved effective in the treatment of leukemias and neuroblastomas. It is a hexadentate compound with three hydroxamate groups that forms a very stable complex of 1:1 stoichiometry with Fe++. Thus, prevention of the formation of ROS. However, DFO has a short plasma half-life and is markedly hydrophilic, which makes it ineffective when taken orally. Thus, it must be administered by continuous, long and painful sub-cutaneous injections. These disadvantages have motivated the scientific community to find iron ligands that are easier to administer even if they are not more effective.

Deferasirox (DFX) (Figure 4), a synthetic tridentate molecule that forms 2:1 complexes with Fe++, is administered orally in clinical treatment of secondary iron overload and has been tested on human myeloid leukemic cell lines. This hydrophobic compound has shown antiproliferative activities in vitro on cell cultures but high concentrations are needed to obtain an inhibitory effect on DNA synthesis. The antiproliferative effect of Deferasirox results from the depletion of iron, ROS induction and the modulation of the metabolism of polyamines, widespread molecules that are essential, like iron, for cell proliferation. Deferiprone (DFP) (Figure 4), approved in the United States for the treatment of thalassemias, also inhibits the proliferation of tumor cells in culture. DFP is a synthetic bidentate ligand of the hydroxypyridinone family which binds iron in a 3:1 ratio at physiological pH with a particularly high affinity for Fe++, however, Fe³⁺ can compete for the metal-binding site of the chelator. Indeed, DFP is selective for trivalent metal cations over dibasic cations. Deferiprone has a shorter half-life and has anti-proliferative and cytotoxicity activity in several human tumor cell lines. According to the literature studies, DFP acts as a pro-oxidant or protective anti-oxidant. The efficacy of this chelator in reducing iron concentration is variable, but is thought to be comparable to that of DFO.

In addition to these three ligands commercially-approved drugs, many others are at various stages of clinical trials. The thiosemicarbazones, led by Triapine (Figure 5), are tridentate chelators that have shown marked antiproliferative activities in vitro and in vivo on a large variety of cancers, acting as RR inhibitors. These compounds can chelate intracellular Fe²⁺ and Fe³⁺ leading to a depletion in iron while also generating ROS responsible for their antitumor effect. There are currently 31 clinical studies worldwide testing Triapine on various cancers, some of which have shown a much greater antiproliferative effect than that of DFO.

Figure 5: Structures of different iron ligands used in clinical trials

The hydrazones are another class of chelators developed following studies carried out with pyridoxal isonicotinoyl hydrazone (PIH, Figure 5). These tridentate compounds form strong complexes with iron similarly to Triapine, except that different atoms are involved in the coordination. Further studies revealed that PIH has greater iron chelation efficacy than DFO but seems to act in the same way by inhibiting DNA synthesis in various cell models.

Tachypyrine is a hexadentate ligand constituted of three pyridine moieties linked to a triminoacyclohexeno scaffold in which chelation occurs via the six nitrogen atoms (Figure 5). Although it has the ability to chelate cations other than iron, studies have shown that its cytotoxic effect is due to iron depletion. In in vitro studies, this chelator has demonstrated a greater cytotoxic activity than DFO. It inhibits ferritin synthesis and leads to apoptosis in a large number of cell lines.

Desferrithiocin (DFT), a siderophore isolated from Streptomyces antibioticus, is a very effective tridentate chelator that can be administered orally (Figure 6). However, its high toxicity results in kidney damage and neurological problems. For this reason, much research has focused on the synthesis of less nephrotoxic analogues. Studies of structure/activity relationships have shown that replacing pyridine by a benzene core (Deferritin) and introducing a
polyether fragment (Deferizazale) considerably reduces the toxicity, even with a twice daily oral administration.36 Deferizazole thus seems very promising and is currently in phase II of clinical trials.37,38

Figure 6: Chelator analogues of DFT

Lastly, another interesting family of chelators contains the 8-hydroxyquinoline moiety. The potential value of this structure in an anticancer strategy is illustrated by the example of Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) (Figure 7). This antibiotic is able to chelate a variety of metal ions, which results in an antiproliferative activity on tumor cells in culture and on grafted tumors in animals.39,40 8-Hydroxyquinoline moiety is also found in the chelator O-Trensox, which has a higher affinity for iron than Deferiprone.41 O-Trensox is a hexadentate ligand that forms a very stable complex of 1:1 stoichiometry with ferric iron by coordination of three phenolic oxygens and three pyridinic nitrogens. It has a much greater affinity for Fe3+ than any other biological metals, but it is also efficient to chelate Fe3+, Cu2+, Zn2+ and Al3+.42 Thus, this lack of selectivity could be a problem and may lead to the deficiency of other physiologically important metals. However, for anti-cancer therapy the risk of mineral depletion may be lesser importance than in the chronic treatment of iron overload. A feature 8-hydroxyquinoline-iron complex is its capacity to redox cycle and the generation of reactive oxygen species. This is reflected in the affinity of 8-hydroxyquinoline moiety for Fe3+ and Fe2+.43,44 Like the objective is the treatment of malignant disease, the induction of oxidative stress may actually be a beneficial feature of an iron-chelator complex, if it contributes to induction of cytotoxic response in the tumor. O-Trensox has been shown to be less effective than DFO in mobilizing the iron of ferritin or of hepatocytes, but more effective in decreasing the level of hepatic iron. This chelator causes iron depletion in cells, which is accompanied by a slowing of cell proliferation associated with the inhibition of DNA synthesis. Given these interesting properties, much research has focused on the development of analogues, particularly by modifying the length of the polyethylene chains carrying the three hydroxyquinoline moieties in order to modulate the hydrophile/lipophile balance. More recently, F. Gaboriau’s team and the Servier Research Institute have investigated the analogue S1 (Figure 7), which has only two hydroxyquinolone cores linked by a diethylbenzylamine chain. Tests carried out in vitro on human hepatocytes have shown an inhibition of DNA synthesis and cell proliferation greater than with O-Trensox treatment. In other words, the chelator S1 has an improved antitumor activity compared with the parent molecule.

Figure 7: Chelators with hydroxyquinoline structures

On the basis of these observations, we are interested in studying new selective Fe3+ ligands based on the association of a chelating hydroxyquinoline moiety and linear polyamines that act as a vector. In fact, a recurring problem in anticancer therapy is the high toxicity of the molecules used, which leads to significant side-effects. For this reason, the specific targeting and delivery of therapeutic molecules toward an organ, tissue or cell has become essential for the treatment of human diseases, especially cancers. In tumor cells, polyamine metabolism, including biosynthesis and uptake, is particularly amplified and the efficiency of the polyamine transport system (PTS) is greatly increased.45 This property of tumor cells is of potential interest for targeting antitumor agents.46 This concept of tumor vectorization by polyamines has been developed to vectorize cytotoxic compounds such as acridine,47 anthracene,48 camptothecine,49 naphthoquinone,50 azepine and benzazepine,51 or molecules carrying boron atoms for boronotherapy,52 and to monitor the intracellular traffic of polyamines toward the cells by using fluorescent polyamine analogue.53-55 More recently, F14512, a novel spermine epipodophyllotoxin conjugate, an inhibitor of DNA-topoisomerase II, has been selected for clinical development in Acute Myeloid Leukemia phase I.54 Such a concept has also been reported in previous studies on the synthesis and the biological properties of a chelating Deferiprone-type moiety linked to spermide.55,56

Thus, we have developed chimeric molecules, which we call “Quilamines”. These compounds, and particularly the most effective molecule HQ1-44 (Figure 8), have a hydroxyquinoline moiety linked in position 2 to a spacer carrying a polyamine of the type spermine and spermidine.57,58

Figure 8: Vectorized ligands: Quilamines and HQ1-44

The polyamine chain plays a triple role: (i) first it acts like a molecular taxi to carry the chelator specifically inside cancerous cells that have an overactive polyamine transport system; (ii) after this targeting, it participates in the coordination of iron via its first nitrogen atom; (iii) then, after being internalized, the polyamine chain can form a complex with DNA and thus increase the antiproliferative activity. The chelator HQ1-44 with homospermidine cargo was shown to be the most selectively transported by the PTS and to be involved in iron coordination. It has a strong affinity for Fe3+ (gFe3+ = 19.4), forming a 2:1 complex at physiological pH, as well as great selectivity toward other metals of biological interest. The good antiproliferative activity of Quilamines has been shown in vitro on several cancer cell lines in a micromolar range of concentrations, as well as weak cytotoxicity and greater selectivity regarding the PTS in the CHO/CHO-MG cell model. Moreover, Quilamine HQ1-44 has little effect on a non-transformed fibroblast line, showing its differential activity between healthy and cancerous cells and, de facto, its great potential for an application in oncology. Proof of the concept was provided by the demonstration of its antitumor activity in animals, comparable to that of cisplatin, the reference anticancer agent. Unlike cisplatin, however, Quilamine HQ1-44 shows no toxicity in immunosuppressed Swiss nude mice.

A study of the antitumor activity of HQ1-44 after PTS activation, by nutritional or drug-induced polyamine deficiency, confirmed the strong potential of this new chelator. A polyamine-free diet, with or without difluoromethyl ornithine (DFMO), an inhibitor of polyamine synthesis, in the
drinking water, was administered to Swiss nude mice xenografted with HCT116 cells derived from human colorectal carcinoma. The joint HQI-44/DFMO action coupled with the polyamine-deficient diet almost halted tumor growth after two weeks of treatment.⁵⁹

Taken together, these results demonstrate that the antiproliferative and apoptotic properties of iron chelators make them promising adjuvant elements in the treatment of certain cancers, particularly those that are iron-dependent such as leukemias, neuroblastomas and breast cancer. Although neoplastic cells require more iron than normal cells, it seems indispensable for a personalized treatment in the future to vectorize the chelators specifically towards tumor cells.

References and notes

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Figure 1: Intestinal absorption of iron

Figure 2: Cellular transport of iron
Figure 3: Schematic representation of possible iron chelators

Figure 4: Structures of different iron ligands used in clinical treatments

Figure 5: Structures of different iron ligands used in clinical trials

Figure 6: Chelator analogues of DFT

Figure 7: Chelators with hydroxyquinoline structures
Figure 8: Vectorized ligands: Quilamines and HQ1-44
**Graphical Abstract**

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