

The design and anticancer effects of experimental and clinically used iron chelating drugs.

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Introduction:

There are many chemicals, biological processes and other factors that are known to cause different forms of cancer. These include environmental, genetic, dietary, metabolic, hormonal and immunological factors, viruses, microorganisms and their products and possible combinations of two or more of these.

Metal ions play an important role in health and disease, including cancer. All normal and neoplastic cells require essential metal ions such as iron, zinc and copper, metal ion containing proteins, and associated metabolic pathways for growth and proliferation [1,2]. However, under certain conditions some essential metal ions such as iron and copper can cause DNA damage through the catalytic formation of toxic free radicals and other oxygen activated products. Liver cancer is well documented in iron loaded idiopathic haemochromatosis patients and in copper loaded Wilson's disease patients [3].

Cancer can also be caused by radioactive atoms used in the nuclear industry such as plutonium and uranium, which emit high levels of toxic radiation [4]. Other toxic metals such as cadmium and nickel are also considered to cause cancer through other mechanisms.

Chelators (Greek: χειλή, claw of a crab) are molecules with specific metal binding sites and high affinity for metal ions. They can be used to modify the chemical, biological and metabolic activity of metal ions and associated proteins or metabolic pathways. Chelating drugs are used for the treatment of more than twenty iron and other metal overloading conditions [5]. Deferoxamine and deferiprone (L1) are used for the removal of iron in transfusional iron loading conditions such as thalassaemia, myelodysplasia and sickle cell anaemia. Penicillamine is used for the treatment of copper overload in Wilson's disease.

In contrast some chelator metal complexes are used for the treatment of cancer eg platinum or gallium complexes, or metal deficiencies eg iron complexes in iron deficiency. Chelator gadolinium complexes are used in magnetic resonance imaging (MRI) and technetium as well as indium in other medical diagnostic techniques [6]. Many naturally occurring molecules such as ATP, citrate and ascorbic acid have metal binding properties and some proteins such as transferrin and caeruloplasmin have high binding affinity for iron and copper respectively and are used for transporting these metals into cells [7]. Hundreds of proteins participating in the

metabolic functions of normal and neoplastic cells bind and incorporate specific metal ions such as iron, copper and zinc, which are essential for their biological activity [3,7].

Many common drugs including the anticancer drugs doxorubicin, mitozantrone, bleiomycin and hydroxyurea, have metal chelating properties and their efficacy and toxicity can be modified by the presence of metal ions and chelating molecules [7]. Within this context, the effect of metal ions and chelators has been investigated in many in vitro, in vivo and clinical experimental models in order to identify molecules and pathways, which can be targeted for the treatment of cancer.

Targeting methods against cancer using chelators

There are several pathways, which can be targeted by chelators and can affect the growth and proliferation of neoplastic cells especially those types, which are more sensitive to metal ions. Chelators that can remove essential metal ions such as iron, copper and zinc from neoplastic cells may have applications in cancer therapy. Similar effects can be observed using chelators or other molecules, which can prevent the uptake of essential metal ions by cancer cells and by targeting the metal transporting proteins or their cell membrane receptors. For example certain types of cancer such as breast, prostate and bladder cancers have an increased expression of transferrin receptors at the cell surface suggesting that they have increased iron requirements [8,9].

A different approach is to target specific iron containing enzymes involved in cell division. In particular, the key iron containing enzyme ribonucleotide reductase, which is involved in DNA synthesis, is the target of many anticancer agents. The anticancer drug hydroxyurea and the iron chelating drugs deferoxamine and deferiprone (L1) have been shown to inhibit DNA synthesis by different forms of interaction with ribonucleotide reductase [10]. It has also been shown that the depletion of iron by deferiprone (L1), deferoxamine and other chelators may affect other targets involved in cancer progression, such as molecules involved in cell cycle control, angiogenesis and metastatic suppression [8].

Another possible target for cancer therapeutics are transcription factors, at least two thousand of which have been identified to be zinc dependent. Specific chelators with high affinity for zinc may also have applications in the treatment of cancer [11]. Many chelators have been tested for anticancer activity. Some of the lipophilic iron chelators such as omadine have been shown in a number of in vitro experiments to have similar anticancer activity to that of the anticancer drugs doxorubicin and mitozantrone [12,13]. Similar effects have been shown by other lipophilic chelators, which have been tested in vivo [8].

Some chelators have been tested in cancer patients, with positive results. Deferoxamine has been used in neuroblastoma and leukaemia patients and triapine is currently being tested in phase II clinical trials [3,14,15].

The antioxidant effects of iron chelators for the prevention of cancer

Free radicals and other damaging oxygen activated products such as the hydroxyl radical, superoxide, hydrogen peroxide and lipid peroxides are usually formed by iron and copper catalytic centers involved in redox reactions and are found in proteins or in the form of low molecular weight complexes with ligands or chelators. Normally the transport, storage and utilization of iron and copper is tightly controlled by specific proteins of iron and copper metabolism. Similarly, the toxic byproducts of free

radicals are continuously eliminated under normal physiological conditions. In addition to the elimination pathways, the control of these toxic byproducts is achieved by antioxidant mechanisms involving low molecular weight antioxidants such as vitamin E, vitamin D, ascorbic acid and lipoic acid or proteins such as superoxide dismutase and catalase [16]. However, under certain conditions the production of excess free radicals and the over-saturation of the antioxidant mechanisms can lead to excess free radical production and toxicity, which can potentially damage all known biomolecules including lipids, proteins and DNA. These effects can subsequently lead to tissue damage. Free radical toxicity cascades, which can cause damage to DNA is considered as one of the major pathways that may lead to cancer [17,18].

There is a major debate at present whether the use of antioxidants can prevent or delay the onset and progress of cancer. Within this context dietary habits involving food components with antioxidant properties or the use of antioxidant pharmaceutical preparations have long been promoted for the fight against cancer [19]. However, despite positive *in vitro* and *in vivo* findings there is no clear clinical evidence that the use of antioxidants have the desired effects. There are several drawbacks in the use of antioxidant vitamins, food derivatives and other pharmaceutical preparations, which are used as therapeutics in the prevention and treatment of cancer. These include the inability of the active ingredient to achieve the desired effective concentrations *in vivo*, the metabolic changes that may render them ineffective, the lipid/water partitioning and tissue compartmentalization *in vivo* and variations in their mode of action. An example in the latter case is that ascorbic acid can act as an antioxidant at high concentrations and as a pro-oxidant at low concentrations [20]. There is increasing evidence that the use of chelating drugs can be more effective than antioxidants in the prevention of free radical toxicity cascades caused by iron or copper catalytic centers. The binding of iron and copper, which may be present in a low molecular weight complex form and /or in related proteins, can be used to minimize free radical toxicity both *in vitro* and *in vivo*. The mechanisms of inhibition and control of free radicals by chelators is similar to that observed by the plasma transport proteins involved in the metabolic pathways for these metals. Some examples are described below.

Dexrazoxane is an FDA approved chelating drug, which is used for the inhibition of the suspected iron induced free radical cardiotoxicity observed in cancer patients treated with the anticancer drugs doxorubicin and daunorubicin [21]. Similar cardioprotective effect has been shown using the iron chelating drug deferiprone (L1), whereas the new iron chelating drug deferasirox had no effect [22,23].

The inhibitory effect of chelators against potential free radical damage and microbial growth induced by iron can also be envisaged following the chemotherapy and radiotherapy treatment of cancer patients. Under these conditions transferrin in many cases becomes fully saturated with iron and can no longer provide protection against microbial growth or free radical damage induced by non-transferrin-bound-iron (NTBI) present in plasma [24].

Many chelators, including deferiprone (L1) and deferoxamine have been shown to inhibit the iron induced free radical damage on deoxyribose, the sugar component of DNA and to have similar effects in a variety of other experimental models involving free radical toxicity [25]. However, some chelators have been shown to have the opposite effect and to increase free radical damage in some of these experimental models. EDTA for example, has been shown to increase the iron induced free radical damage on deoxyribose and deferoxamine the oxidation of haemoglobin to met-haemoglobin [25,26]. The difference in the mode of action of chelators is based on

the redox potential and the stereo-chemical specificity of the iron complexes, which can facilitate or inhibit the catalytic formation of free radicals by the complexed form of iron [25].

Targeting proteins involved in the formation of free radicals and other oxygen activated products is another area where chelators can potentially be used and prevent molecular, cellular and tissue damage. Within this context deferiprone (L1), deferoxamine and other iron chelators have been shown to inhibit cyclooxygenase, lipoxygenase and a number of other iron containing proteins [27]. The level of inhibition by chelators was partly related to their lipid/water partition coefficient. It is envisaged that lipophilic chelators such as L1NAlI can be used to prevent free radical damage in lipophilic cellular compartments and hydrophilic chelators such as deferiprone (L1) to do the same in hydrophilic cellular compartments [28].

Many factors can influence the antioxidant and other pharmacological effects of chelating drugs in vivo such as the rate of metabolism and clearance, the toxicity, the level of iron and other metal ion concentration in the tissue involved etc. Despite these limitations chelating drugs can be considered as the most potent antioxidants intended for clinical use. In particular it is envisaged that deferiprone (L1) may have advantages over other chelating drugs not only because of its effective inhibition of toxic free radical production but also because it can be administered at high doses (75-150 mg/kg/day) and can reach much higher plasma concentrations (0.1-0.5 mM) by comparison to any other known antioxidant pharmaceutical preparations [29].

Future prospects in the design of anticancer chelators with clinical potential

A large number of chelators have been designed and tested for anticancer activity and for supportive therapy in cancer patients using various in vitro and in vivo experimental models. With the exception of hydroxyurea and dexrazoxane, which are routinely used clinically, all other chelators have been tested experimentally and only a few have reached the stage of clinical trials with promising results. It is likely that some chelators, which are currently under development, can be used to target one or more of the pathways, which are involved in different aspects of cancer therapy. In particular, the application of established and commonly used iron chelating drugs of known mode of action and toxicity such as deferiprone (L1), deferoxamine and their combination can increase the prospects of therapeutic applications in cancer patients.

The antioxidant effects of chelators combined with their potential anticancer cytotoxic chelating activity increases their therapeutic index and provides additional advantages over other anticancer drugs. Another prospect of the use of chelators is in combination therapies involving anticancer drugs with a different mode of anticancer activity [6,14]. The prospect of designing chelating pro-drugs, which can be targeted selectively and activated against cancer cells, while sparing normal cells is another approach for clinical application in the treatment of cancer and is currently under investigation.

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