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Synthesis, characterization and *in vitro* anticancer evaluations of two novel derivatives of deferasirox iron chelator

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ABSTRACT

Iron (Fe) chelation therapy was initially designed to alleviate the toxic effects of excess Fe evident in Fe-overload diseases. However, the novel toxicological properties of some Fe chelator-metal complexes have shifted significant attention to their application in cancer chemotherapy. The present study investigates the new role of deferasirox as an anticancer agent due to its ability to chelate with iron. Because of aminoacids antioxidant effect, deferasirox and its two novel amino acid derivatives have been synthesized through the treatment of deferasirox with DCC as well as glycine or phenylalanine methyl ester. All new compounds have been characterized by elemental analysis, FT-IR NMR and mass spectrometry. Therefore, the cytotoxicity of these compounds was screened for antitumor activity against some cell lines using cisplatin as a comparative standard by MTT assay and Flow cytometry. The impact of iron in the intracellular generation of reactive oxygen species was assessed on HT29 and MDA-MB-231 cells. The potential of the synthesized iron chelators for their efficacy to protect cells against model oxidative injury induced was compared. The reactive oxygen species intracellular fluorescence intensity were measured and the result showed that the reactive oxygen species intensity after iron incubation increased while after chelators incubation the reactive oxygen species intensity were decreased significantly. Besides, the effect of the synthesized compounds on mouse fibroblast cell line (L929) was simultaneously evaluated as control. The pharmacological results showed that deferasirox and its two novel aminoacid derivatives were potent anticancer agents.

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1. Introduction

Iron chelation therapy (ICT) has attracted many attentions due to their important roles as chelators (Kontoghiorghes et al., 2005; Nathan and Ann, 2005). Although ICT was first designed to reduce the toxic effects of extra Fe evident in Fe-overload diseases, the novel toxicological properties of some Fe-chelator complexes have shifted their intended application to cancer chemotherapy (Liu and Hider, 2002) (Fig. 1). A significant study has been devoted in Fe chelation therapy to manage systemic Fe overload such as that induced by repeated blood infusions in patients with β–thalassemia (Lovejoy and Richardson, 2000).

The other major effect of overloaded iron is the increase of cancer risk due to the generation of reactive oxygen species (Frey and Reed, 2012; Kell, 2009). Iron can catalyze the production of reactive oxygen species in terms of redox cycling when it is available in a labile, redox-active form (Dixon and Stockwel, 2014; Britton et al., 2002; Liochev, 1999; Burkitt, 2003; Dunford, 2002). Iron and reactive oxygen species are increasingly recognized as important initiators and mediators of cell death in a variety of organisms and pathological situations (Griendling and FitzGerald, 2003; Terman and Brunk, 2006; Kalinowski and Richardson, 2005; Olivieri and Brittenham, 1997).

Some Fe chelators are able to bind and inhibit the redox activity of Fe, making them ideal candidates for the treatment of Fe-overload conditions (Chaston and Richardson, 2003; Richardson et al., 2006). Such ligands are able to prevent excess Fe from participating in Fenton chemistry (Chaston et al., 2003) and inhibit the formation of reactive oxygen species, such as the hydroxyl radical, which initiates oxidative damage. Hence, Fe chelators that remove excess Fe and form redox-inactive complexes provide a useful method of treatment in preventing the toxic effects of Fe overload (Galey, 2001; Halliwell and Gutteridge, 2007). The most important reasons for the use of iron chelators in anti-tumor therapy is tumors developing resistance to chemotherapeutic agents when iron levels rise (Torti and Torti, 2013) and the fact that cancer cells typically require more iron than normal cells to mediate their generally rapid DNA synthesis and growth. Therefore, iron is a target for cancer therapy (Lui et al., 2013).

Two broad strategies in the use of iron chelators in cancer

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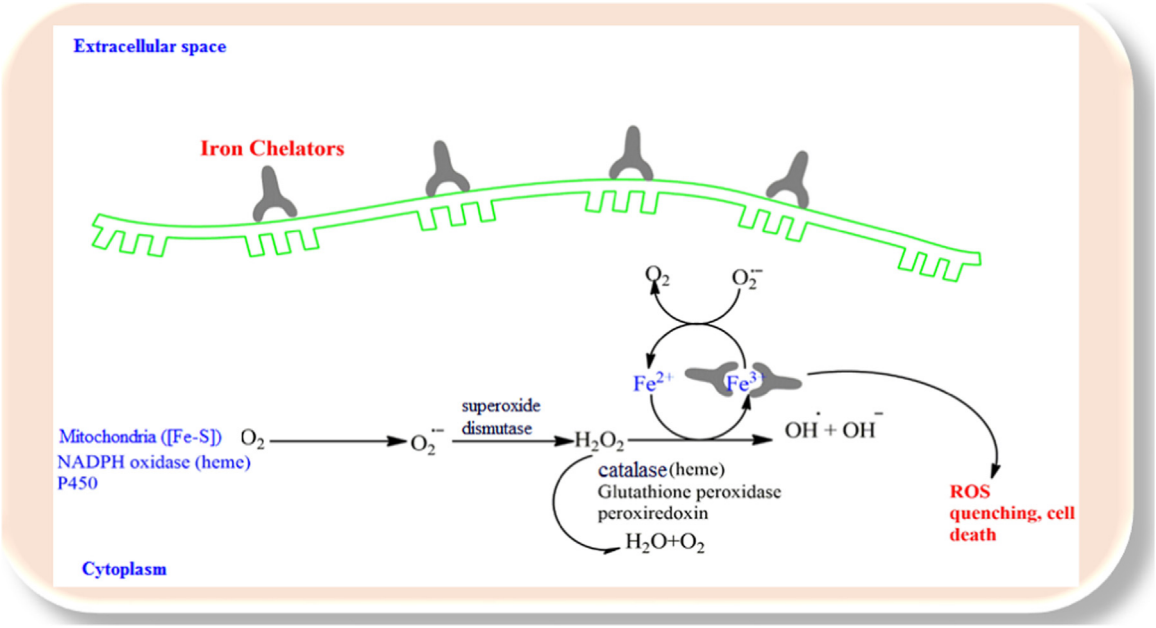

Fig. 1. The role of iron in reactive oxygen species metabolism and iron chelators antiproliferative activity mechanism. The reaction of peroxides with Fe^{2+} to yield soluble hydroxyl (HO•) radicals is referred to as the Fenton reaction. Key enzymes that contribute to reactive oxygen species formation or detoxifiation are shown. Those whose function is iron dependent are colored blue, and the relevant iron species is in parentheses. Clinical iron chelator such as deferasirox are used to enter tumor cells and bind intracellular iron, depriving these cells of this important metal and stop the tumor growth.

treatment have been explored. The first has been to use iron chelators to reduce cancer cells of iron. A second, more recent strategy has been to use chelators that help the redox cycling of iron to generate cytotoxic reactive oxygen species within tumors. Both methods are currently being pursued (Coombs et al., 2012). In this regard, the choice of donor atom play an important role in the ability of a Fe chelator complex to redox cycle and consequently determines its toxicological profile. Those chelators which contains "hard" donor atoms such as oxygen form a stable complex with high-spin Fe (III) and stabilize the ferric state, are not able to form redox-active cycle in the biological conditions (Liu and Hider, 2002) so these class of chelators enter tumor cells and bind intracellular iron, depriving these cells of this important metal and cause to tumor death (Kalinowski and Richardson, 2007) whereas chelators that are able to bind both Fe (II) and Fe (III) have the potential to redox cycle. Iron complexes of chelators applying "soft" donor atoms, such as the sulfur donor atoms, can be enzymatically reduced under physiological conditions. Furthermore, these agents can also form redox-active iron complexes, the resulting Fe (II) can participate in Fenton chemistry, generating reactive oxygen species and oxidative damage. (Chaston and Richardson, 2003). The metal ions key role in both reactive oxygen species production and clearance, have been proved (Jomova and Valko, 2011). Metal ions such as iron readily bind reactive oxygen species ligands, and produce hydroxyl radical in solvent-exposed cellular environments, notably via variations of the simplest form of the Fenton reaction. The actual mechanisms can differ substantially (Kepp, 2012) but commonly aggravate oxidative stress by converting H_2O_2 to much more potent hydroxyl. A simplified type of Fenton reaction, is shown in Eq. (1) (Yu et al., 2012; Liu and Hider, 2002).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^{-} \quad (1)$$

Some examples of these chelators include the commercially available ligands: deferiprone (Ferriprox®), deferasirox (Exjade®) and desferrioxamine (Desferal). These agents which easily coordinate with iron are bidentate, tridentate and hexadentate ligands, respectively (Bendova et al., 2010).

Deferasirox is a chiral, tridentate ligand for Fe^{3+} which has been recently reported as oral iron chelator with efficacy similar to deferoxamine (Steinhauser et al., 2004; Heinz et al., 1999). Early studies examined the anti-cancer efficacy of DFO in neuroblastoma cell lines where exposure to the ligand (60 μM) for 72 h resulted in approximately 90% cell death (Estrov et al., 1987; Nick et al., 2002; Hegetschweiler, 1999). Considering its oral-activity and low toxicity, a number of recent studies have examined the potential of deferasirox as an anticancer agent (Donfrancesco et al., 1990; Chantrel-Groussard et al., 2006; Lescoat et al., 2007).

Further studies found us that deferasirox has been shown high anti-proliferative activity against human hepatoma cells (Vazana-Barad et al., 2013; Nick et al., 2003). In fact, deferasirox was able to reduce cell viability and inhibit the cell cycle at the G_0/G_1 and S-phase in human hepatoma cell line, HUH7 (Epsztejn et al., 1999). Moreover, the anti-tumor effects of deferasirox were more pronounced in hepatoma cells relative to primary cultures of human hepatocytes (Song et al., 2011).

Amino acids like glycine or their corresponding methyl esters alone or accompanying with some bioactive molecules have been proved to show antimycobacterial activity (Stavrakov et al., 2016) and antioxidant effects (Mauriz et al., 2001) It has also been shown that deferasirox combined with glycine can reduce blood lead content in animal models. (Najarnezhad and Asri-Rezaei, 2015). These finding prompted us to synthesize combined deferasirox–glycine or phenylalanine methyl esters and evaluate the possible enhancement effects of them as iron chelating in chemotherapy. In fact, the present study is conducted to compare the efficacy of the deferasirox as a chelator and its glycine or phenylalanine methyl ester derivatives to investigate their anticancer activity against human breast cancer cells (MDA-MB-231), human cervix epithelial carcinoma (HeLa), human colon cancer cell line (HT-29), human leukemia cell line (K-562), bladder cancer cell line (T-24), non-small cell lung carcinoma (A-549), mouse neuroblastoma cell line (Neuro-2a) and mouse fibroblast L-929 cell lines. The results were compared with cisplatin as standard through MTT assay. Also reactive oxygen species measurement was performed using H_2DCFDA to evaluate the impact of iron on the reactive oxygen species concentration and the role of synthesized chelator to

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