Anti-tumor Activities of Four Chelating Agents against Human Neuroblastoma Cells

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Abstract. Background: Iron deprivation may be a therapeutic strategy for cancer. It can be achieved by using iron chelators. In this investigation, anti-neuroblastoma activities of a novel ferric chelator 2LL together with DFO, EDTA and DTPA were evaluated. Materials and Methods: SH-Sy5y cells were cultured at 37°C in 5% CO2/95% air in DMEM containing 10% fetal bovine serum. The cells were seeded in 96-well microtiter plates overnight. Then, chelating agents were added into the wells. After 48-hour incubation, viabilities were measured using the MTT method. Results: DTPA had an IC50 value between 60-100 µM; DFO produced about 40% inhibiting effect at 150 µM; 2LL and EDTA displayed about 10% inhibiting effect at high concentrations. Conclusion: For SH-Sy5y cells, DTPA showed the strongest inhibiting effect, DFO displayed a moderate inhibiting effect, while 2LL and EDTA produced minor inhibition. To develop iron chelators as powerful anti-cancer agents is still a challenging task.

Iron plays an important role in life (1). However, iron is toxic when in excess in the body. Iron chelation is the only effective way to remove excess iron in some diseases, such as β-thalassemia. One of the siderophores (natural iron chelators)(2), desferrioxamine (DFO), that was isolated from Streptomyces pilosus in 1958 by a group at ETH (Swiss Federal Institute of Technology Zurich), has been the only drug for the treatment of β-thalassemia patients (1). However, DFO has some disadvantages such as poor oral activity, short plasma half-life and high cost. Much more effort is needed in order to develop an alternative to DFO (3, 4). Recently, representative syntheses of hydroxamate-type artificial siderophores have been reported (5-8). A chiral tripodal trihydroxamic acid, 2LL (Figure 1), could form a stable ferric complex which showed growth promotion activity against E. coli K 12 RW 193(ATCC 33475) (5). It has been realized that iron deprivation may be a therapeutic strategy for cancer. Iron deprivation can be achieved by using various iron chelators (9). Since Blatt reported the anti-neuroblastoma activity of DFO (10), not only DFO but also other iron chelators have been investigated as anti-neoplastic agents (11-19). However, in general, the development of chelators as anti-cancer agents is still at the infant stage.

Neuroblastoma is the most common extracranial solid tumor of early childhood and is still a leading cause of child cancer-related death (20, 21). In this investigation, the anti-neuroblastoma activities of a novel ferric chelator 2LL, together with DFO, EDTA and DTPA, were evaluated.

Materials and Methods

Ligand 2LL was synthesized according to the procedure previously described (22, 5). DFO (desferoxamine mesylate)(Novartis Pharma AG, Basle, Switzerland), EDTA (Sigma Chemical Co., St. Louis, MO, USA), DTPA (Fluka Chemie GmbH CH-9471 Buchs, Packed in Switzerland), and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)(Sigma Chemical Co., St. Louis, MO, USA) were purchased from the corresponding chemical companies. DMEM (Dulbecco's Modified Eagle Medium) and fetal bovine serum (FBS) were purchased from Life Technologies (Grand Island, NY 14072, USA). The human neuroblastoma cell line SH-Sy5y was kindly provided by Prof. Li Shen (Peking University Health Science Center, China).

Various chelating agents were dissolved in DMEM solution and the pH of the solutions was adjusted to 7.4. SH-Sy5y cells were cultured at 37°C in 5% CO2/95% air in DMEM containing 10% fetal bovine serum (FBS). The cells at 1000 cells/well were seeded in 96-well microtiter plates and left to attach overnight. The next day, a fraction of the solution of chelating agents was added into the wells, respectively, according to the required quantity. Control samples contained DMEM solution only without chelating agents. For a given concentration of a chelating agent, at least 4 wells were tested. The plates were incubated over a 48-hour period at 37°C.

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Figure 1. Structure of chelating agents.
Twenty µL MTT (5 mg/ml) was then added to all wells and the cells were incubated for 3 hours. Viable cells were measured using the MTT dye reduction assay (23).

Results
The effects of the chelating agents EDTA, DTPA, DFO and 2LL on cell viability were evaluated using the human neuroblastoma cell line SH-Sy5y (Figure 2). Under the conditions of this investigation, among these chelating agents DTPA showed the strongest inhibition of cellular proliferation, with an IC_{50} value from 60-100 µM. DFO also displayed a marked inhibiting effect at 150 µM. 2LL produced an approximately 10% inhibiting effect when its concentration exceeded 250 µM. The inhibiting ability of EDTA was similar to that of 2LL.

Discussion
DTPA and EDTA, having similar structures, are both membrane-impermeable chelators. Their sites of action are the extracellular fluid and plasma membrane and within the endosome. However, their abilities to inhibit the proliferation of neuroblastoma SH-Sy5y and hepatocellular carcinoma cells are markedly different. DTPA was the most effective inhibiting chelator, but EDTA had very little effect on the proliferation of these cell lines (17). Blatt et al. (24) also found that DTPA displayed extremely effective inhibition (IC_{50} = 60 µM) of the neuroblastoma cell line CHP 126 (24). DTPA’s anti-proliferative activity may be attributed to its broad coordination abilities to a range of cations rather than specific ferric ion. In addition to strongly binding to trivalent cations, such as Fe^{3+} (log K: 28.0), it also chelates divalent cations, for example, Ca^{2+} (log K: 10.8), Mg^{2+} (log K: 9.34), Fe^{2+} (log K: 16.4) and Zn^{2+} (log K: 18.3) (25). Both Fe^{3+} and other cations, Ca^{2+}, Mg^{2+} etc. are essential for the proliferation of neuroblastoma cells. Compared with DTPA, EDTA has lower stability constants in these cation complexes (log K: Fe^{3+}: 25.0; Ca^{2+}: 10.6; Mg^{2+}: 8.83; Fe^{2+}: 14.3; Zn^{2+}: 16.4)(25). Thus, DTPA may have a stronger capacity to deprive not only Fe^{3+}, but also Ca^{2+}, Mg^{2+} etc.

DFO is a specific ferric chelator, identified in microorganisms. In the presence of other divalent cations, DFO has a high selectivity for ferric ion (log K: Fe^{3+}: 30.6; Ca^{2+}: 10.6; Mg^{2+}: 8.83; Fe^{2+}: 14.3; Zn^{2+}: 16.4)(25). DFO is a membrane permeable ferric chelator. Its moderate anti-proliferative effect on the neuroblastoma cell line SH-Sy5y may be interpreted as a consequence of deprivation of ferric ion, but not other cations, such as Ca^{2+}, Mg^{2+} etc.

2LL is a trihydroxamic acid, which resembles DFO in spite of their different molecular backbones. 2LL has a tripodal structure instead of the linear structure of DFO. 2LL formed a stable ferric complex within the pH range 4.5-10.2. It was shown that the ferric complex of 2LL was more stable than that of Fe(III)-EDTA based on the competition equilibrium between Fe(III)-2LL and EDTA (5, 22). In contrast to DFO, 2LL showed a low inhibiting effect on

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Figure 2. Effects of the chelating agents EDTA, DTPA, DFO and 2LL on cell viability. Cells were incubated for 48 h with chelating agents at various concentrations and viability was assessed using the MTT assay. Results are means of quadruplicate determinations.
neuroblastoma cell SH-Sy5y proliferation, despite its high affinity to ferric ion. We found that 2LL was more hydrophilic than DFO, meaning that 2LL permeated through most cell membranes with more difficulty compared with DFO (4). Probably, this hydrophilic property of 2LL is responsible for its low inhibiting effect of neuroblastoma SH-Sy5y cell proliferation.

The syntheses of hydrophobic compounds with various molecular shapes may provide novel specific ferric chelators with more effective anti-proliferative ability for cancer cells. The development of iron chelators as anti-cancer agents is still a challenging task.

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References