Chapter IV

Discussion and Conclusion

During this thesis, it was shown that Fe(III) undergrew complexation with DFO and yielded a reddish orange stable complex: the so-called ferrioxamine. In this study, the stoichiometry of the complex was 1:1 ferric ion to DFO. The physico-chemical properties of this complex can easily be studied by using spectrophotometric and potentiometric techniques. The complex was stable under light protective conditions at 25 °c for at least 24 h. DFO itself is a weak base molecule at the first ionic constant rate, pKa ($-SH_2O_3/-SHO_3^-$) was 7.2. We designed DFO as the chelator of ferric ions and for this purpose the mesylate moiety should completely be protonated by adjusting the pH of the working solution to 4. Once, the reaction was finished, the pH of the solution was adjusted to 7.25 by using 0.1 M NaOH.

DFO alone is moderately toxic against small cell lung carcinoma (GLC4) compared with pirarubicin, IC_{50} = 10 ±3 nM for pirarubicin and IC_{50} = 30 μM for DFO. The Fe(III)-DFO exihibited cytotoxicity against the same cell line 4 times lesser than DFO alone. This study reported for the first time, that the IC_{50} of Fe^{3+}-DFO was equal to 120 μM. As previously mentioned, the complex form of DFO with ferric ion reduced its cellular toxicity by 4 times. Ferrioxamine thus is potent paramagnetic molecule for use as a probe to monitor labile iron pool (LIP) and/or direct labeling of cells particularly stem cells for stem cell homing, engrafting and functions in the body combined with MR imaging.

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The T1 images of phantoms demonstrated that the T1 signal intensity ordered from high to low was 3.7% agarose gel, RPMI 1640 and water, respectively. The T1 relaxation time of water (3947 ms) was longer than the physiological solution (3290 ms) and 3.7% agarose gel (2381 ms). This suggested that the solutes, such as amino acids, proteins etc… possessed shorter T1 relaxation time than water, and particularly agar, thus they predominantly originated the T1 signal intensity of the solutions. In comparison with T1, T2 relaxation time of water and amino acids was very long (could not be measured by using the studied method) while the agar possessed T2 relaxation time (70 ms) about 43 fold more rapid than T1 relaxation. Among the phantom studied, water originated the maximum T2 signal intensity followed by RPMI 1640 and the agarose gels. A potential use of ferrioxamine as paramagnetic probe for MR imaging was investigated.

Ferrioxamine efficiently enhanced both T1 and T2 image contrast of phantoms. In this study, water, physiologically like serum, RPMI1640 and agarose gel were used as the tissue equivalent phantoms for testing the magnetic susceptibility of the complex. For all series of experiments, water was in excess conditions and it was not the limiting step of the reaction and ferric was complexed and stabilized by DFO. As excepted, the T1 signal intensity that originated by protons relaxation in the presence of Fe^{3+}-DFO complex was almost the same for the phantoms, T_{obs} was dependent upon the concentration of Fe^{3+}-DFO complex. These results supported the hypothesis that the contrast of T1 images when applied the Fe^{3+}-DFO complex was predominately originated by interaction of free water molecules with the ferric-ion. The T1- signal intensity depended upon the dissociation ratio constant of the exchange of water molecules bonded to the complex and the complex concentration.

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These results allowed us to determine the relaxivity of the complex that was equal to 1.05 ± 0.4 $mM^{-1}s^{-1}$. The concentration of the complex that contributed to cause a change in unit of T1-signal intensity can be calculated by equation (2):

$$1/T_{observe} = 1/T_{phantom} + R.C \quad \text{..... (2)}$$

Where $T_{observe}$ and T_{system} was T1 relaxation time of phantoms with and without ferrioxamine, R and C was the relaxivity and concentration of ferrioxamine, repectively.

The results showed that by using ferrioxamine as the MR contrast agent can increase in the sensitivity of proton imaging by micromolar range of concentration.

For T2-signal intensity that originated by a transverse relaxation of proton of water molecules in the presence of Fe^{3+}-DFO complex was biphasic response pattern, at low concentration of the complex (1-5 μM) and short TE. No significant change in T2 signal intensity was observed. However a decrease in T2- signal intensity tends to be measured by using longer TE, but it cannot be measured due to a limitation of the instrument. At higher concentrations (from 1 to 10 mM), the T2- signal intensity was linearly proportional to the concentration of complex. The relaxivity of Fe^{3+}-DFO was 1.17±0.2 $mM^{-1}s^{-1}$. The results indicated that the interpretation of the contrast of T1 image in the presence of Fe^{3+}-DFO should be carefully achieved. The Fe^{3+}-DFO complex should be useful as a T1-contrast agent for Molecular imaging.

Precontrast conditions

T1-images of Wistar rats (TSE, TE 15 and TR 550 ms; see figure 3.12 and 3.13) clearly showed that the fat tissue processed the highest T1-signal intensity (white); proteins-protein complexes possessed medium signal intensity (grey scale); water possessed the lowest signal intensity (black). These signified that the protons

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from biomolecules in the tissue originated the T1-signal and predominately contributed to the tissue image contrast.

Post contrast conditions.

T1-images of Wistar rats (TSE, TE 15 and TR 550 ms) after administering the paramagnetic agent clearly showed an increased in the T1-signal intensity in the tissues such as I the vascular systems, urinary system (including renal cortex and pelvis, ureters and bladder), brains and muscle tissues. However, no change was observed for the fat tissues. These also indicated that in the presence of paramagnetic compound Fe^{3+}-DFO, modification of image contrast was responsible by the mobility of water molecules and after subtracting the T1-signal intensity by the T1-signal intensity of the corresponding ROI of the series without administrating of complex, it could be can be used to calculate the accumulation of Fe^{3+}-DFO in the considered tissues by using equation 2. Indeed, this study provided evidence that the sensitivity of MR imaging of tissue was increased by about 1000 times than those of conventional techniques.

It was thus proposed that Fe^{3+}-DFO might be a suitable probe to conjugate with specific targeted contrast agents for specific proposes of molecular imaging such as for detection of early stage of cancers, for drug response during cancer treatments and for stem cell labeling.

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Conclusion

A suitable MR-image probe, ferric-DFO was synthesized and characterized by spectrophotometric and potentiometric techniques. The Ferric-DFO complex was proposed as a T1-contrast enhancer that dramatically caused an increase in the sensitivity of proton imaging of tissues by 1000 times than those of conventional methods. For further research on pharmacology and toxicological studies, the ferric-DFO complex could be considered (a) as a tissue MR-contrast agent for the study of the urinary system, brain and hearts, (b) as a suitable probe for bioconjugation with biomolecules for specific targeted contrast agents and (c) as a probe for directed labeling of stem cells for stem cell homing, engrafting and function *in vivo*.

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