Chapter I

Introduction

Proton magnetic resonance imaging is a powerful technique and has recently played an import role in molecular imaging of the body's internal structure. Magnetic resonance (MR) images can detect a very small difference of tissue density and thus show the body's internal structure with high resolution. The MR image, derived from the MR signals, depends on the density of the protons in the given tissue, and on the two relaxation parameters of these protons which are referred to as T1 and T2. Recently, the MRI scanner is composed of both imaging and spectroscopy units which allow us to get the body's internal structure and chemical compositions, particularly the metabolites of tissue.

In the human body, the most intense T1 signal is received from fatty tissue due to low concentrations of water whereas tissues containing high concentrations of water, as for example cerebrospinal fluid and edematous tissue, provide a T1 signal of low intensity. Compartments containing high concentrations of proteins, such as the blood stream and muscle tissue, are associated with an intermediate T1 signal intensity. The administration of a paramagnetic ion into a specific compartment will alter the T1 proton relaxation. The introduction of the magnetic field associated with one or more unpaired electrons will alter the interactions between the protons and their environment. As a result, the T1 relaxation time of the protons will be shortened. The magnitude of this change is dependent upon the relative concentration of both protons and the paramagnetic ion.

Paramagnetic ions such as iron, manganese and gadolinium have been utilized as contrast enhancers (Giesel, F. L; et al, 2006; Ebert, S. N; et al, 2007; Merbach, A;

et al, 2001; Caravan, PJ; et al, 2001; Aime, S; et al, 2002) Contrast agents containing ferric iron have been used as an alternative to gadolinium. The paramagnetic nature of ferric iron alone provides significant enhancement of the proton magnetic resonance signal by shortening the T1 relaxation time of protons. However, free ferric ions did not display a stable tend to polymerize and precipitate in an aqueous physiological solution. They are always found in a complex form in the body. In fact ferric ions are necessary for many biochemical pathways of cells and the changes in the cellular iron pool can signify the health status of cells such as by detecting apoptosis, carcinogenesis and progressive cancer (Artemov, D; et al, 2003; Homan, R; et al, 1985; Giesel, F. L; et al, 2006). The development of ferric contrast agents as specific target complexes for MR imaging of the biochemical and physiological function of cells will allow us to visualize biochemical and physiological changes of cells in the body this is known as molecular imaging.

Transferrin receptor (TfR) overexpression in cancer cells

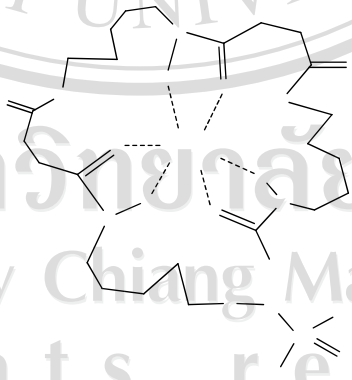
The overexpression of endogenous TfR has been qualitatively described for various cancers (Basar, I; et al 1991, Shindelman, JE; 1981 et al, Sutherland, R; et al 1981 Trowbridge, IS; et al 1981, Trowbridge, IS; et al 1981), presumably due to the malignant transformation of normal cells, and therefore TfR may represent a suitable target for the early detection of cancer. TfR activity in malignant tissue correlated well with the histological grade and pathological stage of the tumour. Patients with low grade superficial tumours showing TfR activity had a higher recurrence rate than those with no TfR activity. It was concluded that TfR activity in low grade superficial bladder tumours is a useful marker for predicting the recurrence rate of

cancer. These findings have suggested a number of possibilities for the application of MRI to monitor disease-specific changes in endogenous TfR expression.

Ferric-Desferoxamine mesylate complex

The feasibility of imaging the TfR also highlighted the need for synthesis of probes with improved sensitivity to image changes in endogenous TfR expression. For these purposes, desferoximine mesylate (DFO-mesylate, for chemical structure see figure 1.1a) was chosen to be used as chelator of ferric ions. In an aqueous solution DFO-mesylate should form a cave and chelated metal ions in particular would form a stable complex with ferric ion (DFO-mesylate, for chemical structure see figure 1b) called ferrioxamine.

(a)



(b)

Figure 1.1 Chemical structure of desferoxamine mesylate and ferric-desferoxamine mesylate complex

O

HN C

Ferrioxamine is a stable complex of ferric iron and deferoxamine, having a binding constant of about 10^{-30} (Hallaway, PE; et al, 1989). The ferrioxamine could be considered as a probe and the mesylate group was designed to conjugate with pharmacological molecules such as by transferrin.

Application of ferrioxamine in MR imaging

Contrast agents based on Mn^{2+}, Fe^{3+}, and Cu^{2+} ions are attractive alternatives (Colet J-M, et al, 1998; Lauffer, RB., et al, 1987; Merbach, A., 2001; Caravan, PJ; et al, 1999; Kupka, T; et al, 1992) because these metals are already present in tissues and there is extensive information available about their human biochemistry, including transport, storage, compartmentalization and excretion mechanisms. Many of the iron-based contrast agents consist of superparamagnetic particles of colloidal iron oxides (SPIO) that cause T2-enhancement (Artemov, D., 2003, Davies, JA; et al, 1999; Wei, J. J; et al 2007). A second type of iron-based contrast agent is ferric mononuclear complexes. In this case the ligand molecule provide coordinative saturation of the iron, avoiding the occurrence of Lewis acid-catalyzed hydrolysis reactions and redox processes (Richardson, Davies and Radüchel, 1999) which are potentially facilitated by free Fe^{3+} ions and are toxic to the body. Furthermore, Fe^{3+} ions must be chelated to avoid precipitation at pH ~7 (Richardson, N; et al., 1999).

Among Fe^{3+} chelators, desferoxamine is a potential molecule used to synthesize MRI based Fe^{3+} contrast agents (Udomuttaracheva, A; et al., 2007, 2008). Due to the size of Fe^{3+} atoms, the distribution and residence time in the tissues should differ and should produce complementary information to that given by Gd-based contrast agents and iron oxide nanoparticles (Wei, J. J; et al 2007; Giesel, F. L; et al,

2006; Artemov, D; et al 2003; Davies, JA et al, 1999; Jacobs, A; et al 1980). Such imaging approaches may not only refine the understanding of the therapeutic mechanism in pre-clinical studies but may also have direct clinical applications.

Objectives

The Application of ferrioxamine conjugated to pharmacological molecule as specific probe for studying TfR expression of cancer cells was a research topic of PCMCB laboratory. This M.Sc. thesis focused only on the ferrioxamine alone. The objectives were

- to determine cytotoxicity of Fe(III)-desferoxamine complex against small cell lung carcinoma GLC4
- to investigate the potential use of Fe(III)-desferoxamine as MRI contrast
- to determine its biodistribution in rats by use of MRI