Part Three

Some Practical Methods/Procedures for Disease Management

Chapter 7

Magnetic Resonance and A Simple Tech for Drug Development

Principle of *In Vitro* Experiments and for *In Vivo* Trials under Food and Drug Administration (FDA) for drug approvals are considered in three Phases, with Phase I the test tube or petri dish experiments (*In Vitro*), Phase II the animal models (small animals such as mice and large animals such as dogs and primates), and Phase III, the most expensive Phase, the human clinical trials. Biochemistry for cell biology conducted in petri dishes can be under meticulous control and can readily be conclusive. They are generally inexpensive. Using small animals such as the mice, for *In Vivo* models can have their test environments under total control and are therefore also not too expensive. They can be sponsored by companies with limited resources. Human trials, particularly those with potential harms, due to the complicated human environment that cannot readily be isolated, must rely upon a large sample size in order to obtain certain definitive conclusion. If the specific biochemistry under investigation can be isolated from the varied human environment, the trials may be conducted as simple as the *In Vitro* experiments and focused with greatly reduced sample size. The key here is to be able to change the atoms of the biochemical reactions under investigation with spin ½ isotopes without altering the chemistry and without invoking radioactivity. Presence of the altered isotopes would provide signals in magnetic resonance and deliver the tagged metabolites i*n Vivo* under simple physical control, or under the Simple Tech alternative, which is the theme of this Chapter.

FDA Approval, A Messy Tech and A Messy Business

Medical fields are often far more empirical than physical sciences. They generally are too complex to be organized into simple analytical terms. Nevertheless, the use of non-radioactive isotopes under NMR (nuclear magnetic resonance) spectroscopy or fMRI (functional magnetic resonance imaging) may provide a major tool, the simple tech tool, to initiate science-based biotech developments. They can include certain disease diagnostics and disease therapies as well as help simplify the well known messy tech, the FDA approval.

Current FDA approval, at a price tag of approximately two billion dollars for a major drug, is beyond the financial resources of most biotech concerns, and thus renders them to work only on the early stages of drug development and leave the most expensive clinical trials, the Phase III activities, to only the major pharmaceuticals. *In vitro* (Phase I) analysis and animal studies (Phase II) alone are not inexpensive, although many human diseases simply do not have an animal model. A path not well publicized for the FDA approval is to seek assistance from the Medicare Program for those drugs that benefit seniors.

Under Medicare for senior citizens, most medical expenses are covered by the U.S. federal government. In addition, certain illnesses and their treatment developments that are particularly sensitive to seniors can be supported as part of the Medicare program. The inclusion of treatment developments was initiated by the Clinton administration under the name of "deemed statistics". In this deemed statistics, expensive FDA approvals for possible novel treatments for Alzheimer's disease and type II diabetes, for example, could most likely be covered by the Medicare program in order to benefit seniors in the States.

For clinical trials, the "no harm" aspects alone could involve statistically each and every organ in the body and if fetus and infants are involved, the testing periods must be extended to a much longer duration. Statistically, the standard deviation σ with 66% accuracy is, $\sigma=N^{1/2}/N$, with N the sample size. This gives N=100 for 10% at one σ accuracy, N=10,000 for 1%, etc. For a major drug, using 10,000 patient-organs at a price tag of thousands of dollars each, this no-harm aspect alone would run well beyond the resources of most biotech companies, and therefore they would focus only on the "Efficacy" aspects with trials with animal models if available, and leave the full blown trial to only the majors. There are also "orphan" drugs, mostly for cancer patients where the "no harm" aspect can be waived.

Cell biologists like to control the cellular process using the interference of RNA that directs peptide/protein production. As DNA forms a double helix, RNA can form triple

strands, and can neutralize un-wanted RNA single strands with the triple strand arrangement. Angus Hepburn and I did a huge, one inch thick patent issued 10 years ago, and we were pleased to find that the 2006 Nobel Prize in Medicine by Fire and Mello for the 1998 discovery was on the same topic of controlling cellular process through the neutralization of cellular RNA, although their method was different from ours. They are working in the cell biology field, but we were speculating from afar.

Functional MRI (fMRI) for Brain Hemodynamics

In the brain hemodynamics utilizing an increase of blood flow to an area under neural stimulation. It causes a local change in deoxyhemoglobin, because the increase in blood flow is below a corresponding increase in the oxygen extraction. The deoxyhemoglobin is paramagnetic, whose change services as an endogenous contrast enhancing signal for fMRI. This imaging is highly useful to guide surgical procedures of the brain. The function-specific MRI with endogenous enhancement signal can also be very helpful in identifying the efficacies of various psychological drugs by visual image of their functional impact, boxel-by-boxel (3-D pixel) to all regions of the brain. Some friends of mine play an intense game of "Go", a kind of chess favored by the Orientals. During the game, they would be "pale as the ghost" as claimed by their wives. This change of deoxyhemoglobin in certain regions of the brain under an intense mental stress is a good example of "paleness" to be localized visually under fMRI.

Focusing on the quality of neural transmitters and neural metabolic functions of the brain, a great variety of psychopathological diseases can also be evaluated under the kinetic MRI, whose brain images are taken after a dose of exogenous intensifying agent is injected to obtain an enhancement signal for MRI, similar to the use of injected Gd-compounds. The exogenous molecules could be particularly effective to provide organ-specific information with localized identification of drug functions and their metabolites.

Now consider a simple tech to help develop the drug approval from FDA. Since our body performance is very complex with key functional parameters varying from one person to another. In order to follow these parameters that can be enhanced or modified by a particular compound of drug without invoking unintended changes to other parameters/organs could be exceedingly difficult. If we can identify the desired organ specific signal for any drug and its metabolites

as tags, much of the unrelated "noises" for a large number of persons over time that are normally averaged out statistically can be avoided under the organ-specific chemistry tagged with *in situ* monitoring.

Magnetic Resonance for Spin ½ atoms

Magnetic resonance imaging (MRI) has evolved from static imaging to dynamic evaluations. In static imaging, particularly for the brain, noninvasive imaging has provided much clinical use. Using an enhancement molecule Gd-DTPA, which is one of five FDA approved Gd-compounds, whose leakage from the body fluid a few minutes after injection is used for dynamic imaging. The leaking fraction to the neovasculature, which is generally tumor-specific has a longer clearing time as compared to the normal tissues, and therefore its imaging has become the gold standard of locating the metastatic sites.

The spin of the nucleus, if any, would line up under a magnetic field, and flip by a radio frequency (RF) pulse in resonance with the nuclei, which will decay (flip back) after a short duration and give out a flipping RF signal. The magnetic alignment uses field gradients in each of the 3-D magnetic direction so that only a precise spatial point would provide resonance with the RF, thus spatial imaging location. By varying the magnetic field as well as the RF systematically, assisted by computer control, the MRI becomes an extremely usual diagnostic instrument. MRI capabilities were developed from computerized 3-D compilations of NMR, which was pioneered by Purcell. In this chapter I will introduce two unconventional uses of NMR and MRI, with one related to stable isotopes having a hydrogen-like nuclear spin, and the other utilizing the Gd-based contrast agents for X-rays as well as for MRI as the enhancement molecule.

Except for hydrogen, most elements in our body have spin zero which cannot be aligned with a magnetic field. If an agent containing non-radioactive isotope with hydrogen-like spin is presented to the body exogenously, it can readily be identified under NMR or MRI. The key issue is that while atoms with different isotopes can have different spin for magnetic resonant reactions, they have identical chemical properties and retain the same drug potency. That is, we are preparing a "twin" for the sole purpose of evaluation *in vetro* as well as *in vivo*.

Cellular material such as RNA, DNA, peptides and proteins are composed of

very light atoms mostly with spin zero except for hydrogen at spin ½. Using MRI, the hydrogen-based signal provides mainly the H_2O and lipids presence in various tissues. Using MRI of the brain as an example, it can distinguish white

matter, gray matter, the void, the skeleton, the bone marrow, etc. in spatial distribution and provide much useful clinical evaluations unintrusively. The static imaging of MRI evolved into dynamic transport where metabolic uptakes and neural transmitters became the targeted materials, and they are also considered under "functional MRI" or fMRI.

From the cellular composition of light stable atoms such as carbon, nitrogen

and oxygen, C-12 has spin zero, but C-13 has spin ½; N-14 has spin zero, but N-15 has spin ½; O-16 has spin zero, but O-17 has spin ½. These three isotopes, C-13, N-15 and O-17 all have hydrogen-like spin, and can be analyzed like hydrogen under MRI or NMR spectroscopy if present in the tissue. Since the natural abundance of these stable isotopes is very rare, a small dose of them, can already yield their dynamic distribution or function. Now suppose we alter the atoms from spin zero to spin ½ for certain compounds as tags without changing any chemistry, the fMRI can be extended into the whole body for metabolic uptakes, for neural transmissions, for fluid transports and other dynamic balance or unbalance. Drugs, with altered atoms without altered chemistry, can be evaluated organ-by-organ without resort to huge statistical sampling and equip medical research with tools commonly used in physical sciences.

There are over 10,000 MRI instruments installed in the U.S. and by adding a magnetic coil to follow C-13 or N-15 or O-17 or some combinations of them, it is not an expensive endeavor. The key issue here is the availability of theses stable isotopes. These isotopes can form the tag molecules for a variety of drug compounds, including complicated chemistry for drugs derived from plants, for example, spin ½ isotopes can be fed for the whole plant as well.

Tags for Epi-Genome

Of our 32,000 genes in each cell, vast majority of them are not activating at any one time as their DNA are "methylated". Most of the methylation are tissue-specific and are inherited. Certain methylation/demethylation, for our immune functions for example, are driven by some external needs of the body. The gene MGMT, for example, would demethylate other genes, while the drug Temolozomide would methylate many active genes to terminate their transcription, some times including the MGMT gene.

While our genome are highly stable throughout our life, our epi-genome that controls the genetic functions are less stable and could be manipulated by

external activities such as pharmacological influence. That is, the tags indicating epi-genetic alterations could be the primary indication under the novel spin ½ compounds such as $^{13}CH_3$ for the fMRI or NMR evaluations.

Similar to molecules of the methylation group, a far more important group of molecules that could impact the genetic transcription are the molecules which could condense or loosen the histone particles. As the DNA under double helix is winded between the histones linked by lysine residues like a spool of thread, the lysine residues include acetyl group, whose removal by deacetylase would condense the histones and silence much of the DNA transcriptions. This inhibition has been the focal attention of many food supplements and drug developments. The inhibitor include, for example, the Zinc-based catalytic linkages, the hydroxamic acids, and many other enzyme inhibitors as well as a large variety of non-histone transcription factors and co-regulators that could be modified by the acetylation functions. In general, a condensed stringing of histones would imply less room for DNA transcription and a loosened stringing allows more room for genes to be active for transcription. That is, these molecules associated with histone structures could be the most useful tags made of spin ½ atoms without altering the tag chemistry in order to be analyzed by the fMRI or NMR studies.

Gd-DTPA for X-Rays

While conventional X-ray generators cannot provide the laser-like X-ray photon beams, the NanoRay tube (Chapter 4) can do exactly that. Gadolinium has the highest diamagnetic moment and Gd-DTPA for example, is a FDA approved compound for MRI use. Gd-DTPA cannot generally provide sufficient signal for X-ray imaging. Gd is heavy enough to interact with hard X-ray photons for its K-absorption at over 50 keV, photons hard enough to pass through all parts of human body. Now use a dual X-ray beam with one at just above the Gd's K-edge (Kα of Tm) and another beyond the K-edge (Kα of Ta). They form two similar images except for Gd, and a digital subtraction of two images gives the Gd-specific image.

In addition to Gd-specific imaging, the Kα of Tm that initiates an inner shell ionization of Gd can induce a mega-Gray ionizing does at extremely local dimension, or the *in situ* dose for certain disease management, (Chapter 9).

The seminal work of nuclear magnetic resonance was initiated by Purcell. I once used his electromagnetic textbook for an undergraduate class. When my son took the EM course, he could not get Purcell's EM book and my wife and I

search through New York City with me droving while she hopping in and out of bookstore one after another, found a copy, and happily shipped to our son. But the next day my wife found the same textbook in our basement because I had used this same text for six summers.

"How could you not remember." she was obviously terribly annoyed.

"I used the Berkeley Series for summer classes, but Purcell was a professor from Harvard." I answered.

"The book we bought and yours are identical." She continued.

"Well, Purcell died long ago. The cover of the book we bought is bright pinkish while the cover of mine in the basement has faded into pinkish white." This was my last defense, and she wouldn't talk to me for several days.

A friend of mine at the Veteran's Hospital at UCLA first used NMR to resolve nails on a wooden block to show in principle the viability of MRI. When the Nobel for medicine on MRI went to two mathematicians who developed the analytical algorism, he was rather disappointed. MRI has indeed advanced in an astonishing speed. Almost all the magnetic coils are now super conducting, from 0.5T (Tessler or 10^4 Gauss) to as high as 8T with FDA approval, and they can be produced at a relatively low cost. The image analyses are conducted by ever more powerful computers at relatively low cost. As the magnets gets more powerful, the instrument can now include space for certain surgical procedures. The image resolution has been improved, from cm-plus to the current mm level that can compare to the resolution of a few line-pairs per mm in conventional X-ray images. The two forms of image, of course, refer to entirely different subjects; MRI is for the H_2O concentration while the X-rays is for differential tissue densities. Another development of MRI is the use of heavy diamagnetic nucleus. In addition to the proton's spin as nucleus of the hydrogen atom, Gd as caged under DPTA molecule has become a powerful contrast agent for fMRI, which is different to the static images provided by the hydrogen or H_2O. Many light nucleuses, such as $^{13}CH_3$ for methylation or ^{17}O-acetyl for acetylation for the epi-genomic changes, having the same spin as hydrogen would provide a much weaker signal than protons with respect to their spin-flips. But as the fMRI technology marches to ever higher T, analyzed by ever faster and smarter computers, using the stable light isotopes to tag drug molecules could join the mainstream fMRI service for dynamic uptake studies.

Stories on Isotope Work

Over a decade ago I met a congressman Joe Skin of New Mexico State. He asked me whether if there was any technology that could speed up the billion-dollar (at that time) price of the clinical trials necessary for FDA approval. He was the subcommittee chairman for FDA and if there was any technology to simplify the approval process, he would gladly support it. I told him that the western drug development as we know it is really more an art than a science. What we need to know is specifically the metabolic distributions and their functions of the compound over various tissues and organs. We use clinical trials with large sample size because we could not "tag" the biochemistry *in vivo* (in the body) and must use large sample size to neuturize unrelated individual events or functions. He asked me how could we tag them. I answered that we have isotopes to not only tag them, but also image them for their functions and distributions. Take MRI as an example; there are already 10,000 instruments in operation in the U.S. that could image the spin-flip of hydrogen, which exists mostly in water H_2O, and provide a great deal of tissue information without surgery. Similar to hydrogen with "spin-½", other stable isotopes C-13, O-15, O-17, N-15 etc. also have spin ½ exactly like hydrogen, and they can also be observed under the existing MRI instruments with detector coils modified for the target element(s). We could change atomic isotopes of the drug compound without altering their chemistry and without making them radioactive (radioactive singles can only emit once in a particular signal while the non-radioactive isotopes can be activated under a magnetic field repeatedly), the drug and its metabolites can be followed precisely to each and every organ as well as their biochemical functions, not just a reading of static presence. This process can now be studied scientifically instead of compiling into a large database in a randomized clinical trial. Joe was excited and asked me why was it not implemented. I told him that it was mainly due to the high cost of isotopes. At that time, I was co-sponsoring a Ph.D. thesis at Cornell Medical College involving the metabolic uptake of glucose in the brain using C-13 isotope with functional NMR for analysis. Because of the high cost of C-13, the mice we used were as small as a thumb. To enrich C-13 to 60% from its natural abundance of 1.1% for example (60% would give a good signal from the background), it already cost over one hundred dollars per gram, and as many small mice are used in the study, the material cost of isotopes alone was getting beyond the budget of a doctoral thesis. Isotope enrichment was notoriously inefficient. I told Joe that I have a few schemes that are far more efficient, and if the cost of enrichment could be reduced by a factor of one hundred or more, we can design all the drugs isotopically in order to evaluate them under NMR or fMRI and can drastically alter the procedures of clinical trials. Joe Skin asked me if I would develop such technology in his congressional district in New Mexico. I said yes and that began a long collaboration and friendship. The

production of light stable isotopes has commenced, unfortunately, after Joe's death a few years ago.

CHAPTER SEVEN REFERENCES

On the fMRI

"The Future Role of fMRI in Medical Applications" by Medical Center of the Columbia University Web. "Program for Imaging and Cognitive Sciences.