2-Nitroimidazole as Potential Nanoprobe in Hypoxia Imaging by MRI/PET

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ABSTRACT

2-Nitroimidazole is radiosensitizer and serves as imaging contrast agent by 19F- and 31P MR methods. Despite the success of mesonidazole and SR-4554 compounds, very little is known of its **hepato-cytotoxicity** if it can be potential hypoxia imaging marker and non-toxic drug. Authors established the low oxygen, low energy metabolic ratio and gluconeogenesis in nitroimidazole induced hepatocytes and Kupffer cells leading to apoptosis[1]. Several new nanoparticles of nitroimidazole have emerged: nitroimidazole CI-1010, alginate-, silver-nanobullets as nanoparticles in imaging applications. Emerging 18F/19F double labeling techniques further give hope of MRI/PET imaging by using 18F/19F-FETNIM (erythronitroimidazole). Present art exists: 1. 18F/19F -1[2 fluoro-ethoxy-methyl- or -1[2 fluoro-hydroxymethyl-ethoxy-methyl-2- nitroimidazole can be choice of hypoxia MRI/PET imaging contrast agents; 2. availability of Hyoxyprobe™ and KU-2285 has enhanced the sensitivity of hypoxia quantitation; 3. copper labeled diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) selectively bind to hypoxic tumor cells. Cu-ATSM PET can be modality to image tumor hypoxia and contribute to the PET signal; 4. Improved compartment modeling analysis separates tumor tissue time-activity of nanoparticle signal into intravascular and extravascular components; 5. the dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), blood oxygen level dependent (BOLD) and FLOOD T2* imaging, and the measurement of lactate by MR methods serve as hypoxia surrogates and provide the pO_2 status of the tumor. The fusion of NMR and PET images generates stereotactic marker template of tissue. In conclusion, nanoparticle enhanced multimodal MRI/PET diagnostic sensitivity serves hypoxia events better.

Key words: hypoxia, nitroimidazole, MRI/PET, nanobullets

2 INTRODUCTION

2-nitroimidazole hypoxia markers show considerable promise for clinical use in detection of hypoxia. Several 2-nitroimidazoles analogs have been described. The bioreductive metabolism of 2-nitroimidazoles provides a way of labeling hypoxic cells in vivo but the cytotoxicity of nitroimidazole is still controversial [1].

Its increasing use as radiosensitizer and 18-F labeled compounds has demonstrated its potential as imaging contrast agent for *in vivo* positron emission tomography (PET).

Table 1: The cytotoxicity of nitroimidazole on liver cell energy and drug metabolizing enzymes.

Hepatotoxicity of nitroimidazole	Hepatocytes	Kupffer cells
Pathological	Swollen	Hyperplasia
	Necrosis	Phagocytosis
	DNA breaks	Anisonucleosis
EM	Bizzare-Mitochondria	
	Glycocalyx-ve	Rough EM
ELISA	High titer	
IHA	High Agglutination	
Drug Metabolizing Enzymes:		
1.Alkaline Pase		++++
2.Glucuroinidase		+++
3.Lipase		++++
4.NADPH Oxidase		+++
5.Diphorase		+++
6.Acid Pase		++
7.Nitro-Dehydrogenase	++	++++
Energy Metabolizing Enzymes:		
1.PFK		
2.Pyruvate Kinase		+
3.Cytochrome P450R	++	++++
4.SOD	+	+++
5.GSH	++	++++
6.HMP Shunt	+	++
7.Respiratory Burst	+++	+++

In tumor oncology, tumor hypoxia is common in solid tumors due to oxygen insufficiency and disorganized tumor vasculature. Hypoxia drives genetic instability and resulting tumor progression. 2-nitroimidazole probe may serve as therapeutic bioreductive agents, anti-angiogenic/anti-vascular therapies and hypoxia-targeted therapy such as radio- or chemotherapy in human cancers. Recently, design, validation, preclinical development of a fluorinated 2-nitroimidazole, N-(2-hydroxy-3,3,3-trifluoropropyl)-2-(2-nitro-l-imidazolyl) acetamide (SR 4554, CRC 94/17), was designed for the measurement of tumor hypoxia using magnetic resonance spectroscopy (MRS), imaging (MRI) and positron emission tomography (PET).

Primarily, nitroimidazole probe design goals were: (i) a nitro group with appropriate redox potential for selective reduction and binding in hypoxic tumor cells; (ii) hydrophilic/hydrogen bonding character in the side chain to limit nervous tissue penetration and prevent neurotoxicity; and (iii) three equivalent fluorine atoms to enhance MRS/MRI detection, located in a metabolically stable position.

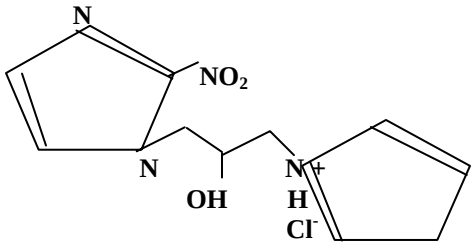

Figure 1: The structure of nitroimidazole HYDROXYPROBE^{TM-1}probe to image hypoxia.

In present study, nitroimidazole is shown as hypoxia marker with role of NADPH:Cytochrome P450 Reductase in guinea pig liver cells and its other utility as MRI/PET contrast as shown in Figure 2.

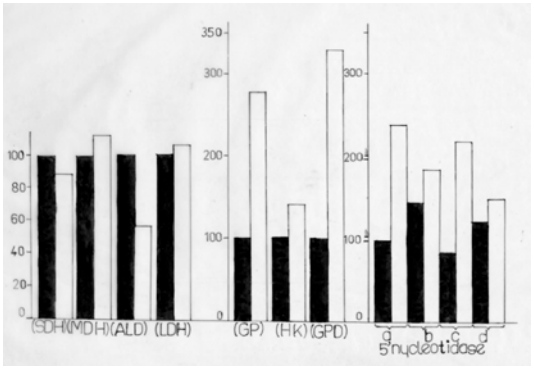

Figure 2: The effect of nitroimidazole of NADH:Cytochrome P 450 Reductase, several other lysosomal enzymes in liver cells [*].

We report the preliminary results of metabolic reduction of SR 4554 by guinea pig liver microsomes and dependence on oxygen content in liver cells, with a half-maximal inhibition at 0.48 0.06%. SR 4554 compound showed nitroreduction in hypoxic liver cells but none in normo-oxic cells in vitro (see Figure 3). Quantitative MRS was used to assess the retention of 19F signal in liver cells. The 19F retention index (FRI; ratio of 19F signal levels at 6 h relative to that at 45 min) ranged from 0.5 to 1.0 for breast tumors. Finally, whole body 19F-MRI in rat demonstrated that SR 4554 and related metabolites localized mainly in tumor, liver and bladder regions. A selective MRS signal was readily detectable in tumors at doses at least 7-fold lower than those likely to cause toxicity in rat. We conclude that proof of principle is established for the use of SR 4554 as a non-invasive MRS/MRI probe for the detection of tumor hypoxia. Based on these promising studies, SR 4554 has been selected for clinical development.

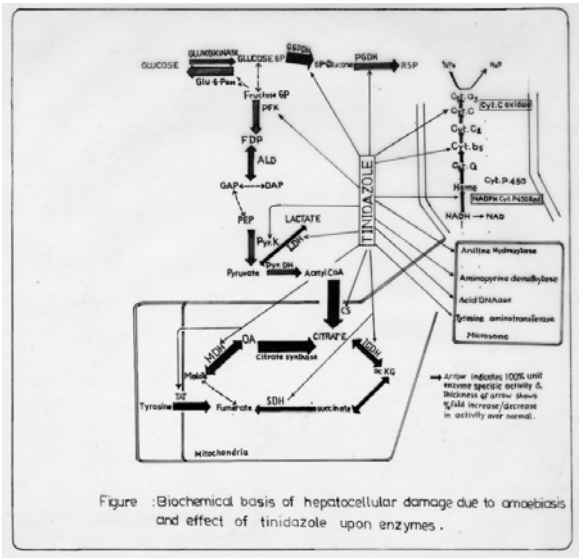

Figure 3: Sketch of nitroimidazole effect on hypoxic liver cells [*].

3 EMERGING NITROIMIDAZOLE AS MRI/PET NANOPROBES

Recently, interest is developing to use nitroimidazole as potential tumor hypoxia marker using fluorescent labeling. Fortunately, double lebeling techniques have emerged as 18-F/19-F labeling of nitroimidazole analogs. The idea is imaging and measuring low oxygen content (hypoxia) in tumor cells by 18-F sensitive PET and 19-F sensitive MRI techniques. The double labeled nitroimidazole probe serves the purpose of low oxygen (hypoxia) marker, imaging and therapeutic monitoring the antitumor drugs. Recently, it is becoming routine art of

metabolic and morphometric imaging by using MRI/PET techniques[2-23].

4 NITROIMIDAZOLE ANTIBACTERIALS

Nitroimidazoles are proven antibacterial compounds. The author established nitroimidazole as best single dose antibacterial chemotherapy compound for hepatic amebiasis by demonstrating its effect on Entamoeba histolytica intracellular metabolism [1].

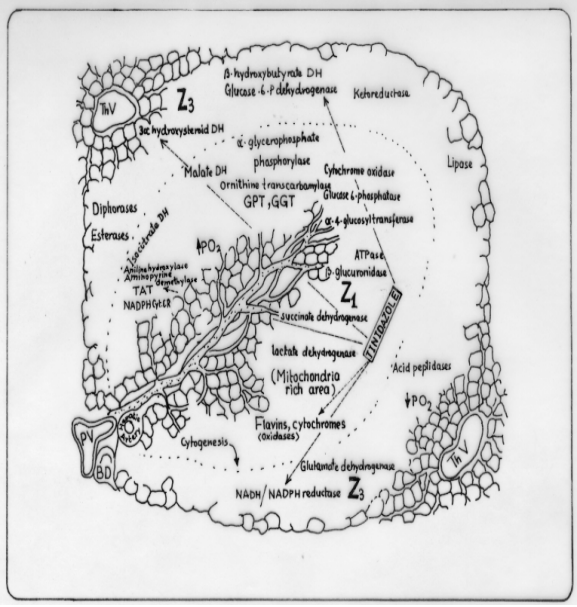

Figure 4: The sketch of nitroimidazole effect on liver intracellular metabolism[*].

Recently, silver bound imidazole compounds have got attention as antimicrobial agents such as Staphylococcus, Escherichia coli species.

'Silver bullets' in antimicrobial chemotherapy: Synthesis, characterisation and biological screening of some new Ag(I)-containing imidazole complexes [13].

Several Ag(I) complexes containing imidazole and derivatised imidazole ligands were synthesised and $[Ag_2(imH)_4](salH)_2$ (imH=imidazole; $salH_2$=salicylic acid), $[Ag(MeNO_2imH)_2]ClO_4 \cdot H_2O$ ($MeNO_2imH$=2-methyl-5-nitroimidazole) and $[Ag(apim)]ClO_4$ (apim=1-(3-aminopropyl)imidazole) were structurally characterised. In vitro tests against pathogenic bacteria and fungi revealed that the complexes possessed significant antimicrobial activity.Simple synthetic routes are given to the Ag(I) complexes, $[Ag_2(imH)_4](salH)_2$ (imH=imidazole; $salH_2$=salicylic acid), $[Ag(MeNO_2imH)_2]ClO_4 \cdot nH_2O$ ($MeNO_2imH$=2-methyl-5-nitroimidazole; n=1, 3), $[Ag(NO_2im)]$ (NO_2imH=5-nitroimidazole) and $[Ag(apim)]ClO_4$ (apim=1-(3-aminopropyl)imidazole). X-ray crystal structures of $[Ag_2(imH)_4](salH)_2$, $[Ag(MeNO_2imH)_2]ClO_4 \cdot H_2O$ and $[Ag(apim)]ClO_4$ were obtained. The new Ag(I) complexes and related known Ag(I) imidazolates were screened for their growth inhibitory effects (in vitro) against the pathogenic bacteria methicillin-resistant Staphylococcus aureus (MRSA) and Escherichia coli and also the fungal pathogen Candida albicans. $[Ag_2(imH)_4](salH)_2$, in comparison to the prescription drug silver sulfadiazine, had significantly better anti-bacterial qualities, whilst against the fungus C. albicans it was 47 times more potent than the marketed drug ketoconazole.

Recently nitroimidazoles were used as nanodrugs W/O/W multiple emulsions containing nitroimidazole derivatives for vaginal application [

5 NITROIMIDAZOLES AS RADIOSENSITI-ZERS

The nitroimidazole radiosensitizer CI-1010 ((R)-alpha-[[(2-bromoethyl)-amino]methyl]-2-nitro-1H-imidazole-1-ethanol monohydrobromide) causes selective, irreversible, retinal photoreceptor apoptosis in vivo [15]. The mouse 661W photoreceptor cell line was used as a neuronotypic model of CI-1010-mediated retinal degeneration. Exposure to CI-1010 for 24h induced apoptosis in 661W cells, as determined by ultrastructural analysis, agarose electrophoresis and analysis of TUNEL-positive nuclei. CI-1010 caused a loss of viability in 661W cells, as measured by the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). A clear link was established between the onset of apoptosis and activity of caspase-3 and caspase-8, prior to poly[ADP-ribose]polymerase (PARP) cleavage. Pretreatment with caspase inhibitors, ZVAD.fmk or DEVD-CHO, prevented morphological changes in most CI-1010-treated cells. Evaluation of mitochondrial inner membrane potential (Deltapsi(m)) in live 661W cells using the fluorescent dye, tetramethylrhodamine methyl ester revealed retention of (Deltapsi(m)) until after caspase activation. Absence of cytochrome c in the cytoplasm in treated cells further supports the hypothesis of a mitochondrial-independent mechanism of cell death. Significant increase in DNA crosslinks observed in 661W cells correlates with induction and phosphorylation of p53 at multiple serine sites. Cell cycle analysis of 661W cells reveals a G(2) arrest in response to CI-1010-induced DNA damage and neuronal cell death. Increased protein expression of Bax, Fas, and FasL, concomitant to the loss of Bcl-xL in treated 661W cells may be modulated by p53. This report evaluated *in vitro* mechanisms of CI-1010-induced cell death in photoreceptors and provides evidence in support of a p53-linked activation of caspase-3 in response to DNA damage caused by CI-1010.

6 NITROIMIDAZOLES AS CARDIAC HYPOXIA MARKERS

In recent past, nitroimidazole was identified as potential cardiac hypoxia marker due to its easy binding with Technitium 99 and easier use in nuclear medicine [16-23]. However, cardiac hypoxia and ischemia remained potential physiological conditions of myocardium to answer by imaging. In that direction, tritium, fluoro-labeled misonidazole imaging probes were reported as potential candidates. However, nitroimidazole probes gained popularity in last decade.

Table 4: The nitroimidazoles as cardiac hypoxia markers.

Probe	Application (reference)
^{99m}Tc-NIM	Myocardial ischemia (17)
^{99m}Tc-NIM	Myocardium Hypoxia(18)
BMS-181321	Myocardium Hypoxia(20)
^{99m}Tc-NIM	Myocardium Hypoxia(22)
Bis(amino-phenol)-NIM	Myocardium Hypoxia(23)
Iodovinylmisonidazole	Myocardium Hypoxia(24)
18F-fluoromisonidazole	Myocardium PET(26)
18F-fluoromisonidazole	Myocardial ischemia (27)

In conclusion, doublelabeled 18F/19F -1[2 fluoro-ethoxy-methyl- or -1[2 fluoro-hydroxymethyl-ethoxy-methyl-2- nitroimidazole can be choice of hypoxia MRI/PET imaging contrast agents. The availability of Hyoxyprobe^{TM} and KU-2285 has enhanced the sensitivity of hypoxia quantitation. The copper labeled diacetyl-bis(N4-methylthiosemicarbazone) (Cu-ATSM) selectively bind to hypoxic tumor cells. Cu-ATSM PET can be modality to image tumor hypoxia and contribute to the PET signal. Improved compartment modeling analysis separates tumor tissue time-activity of nanoparticle signal into intravascular and extravascular components. The dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), blood oxygen level dependent (BOLD) and FLOOD T2* imaging, and the measurement of lactate by MR methods serve as hypoxia surrogates and provide the pO_2 status of the tumor. The fusion of NMR and PET images generates stereotactic marker template of tissue. Nitroimidazoles serve both antibacterial and imaging probes.

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