Mechanism of Nitroimidazole Coated Magnetic Nanoparticles Embedded in Micro-organisms as Delivery Systems

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ABSTRACT

Avirulent Bacteria and parasites act as "tiny missile like carriers" capable to carry the magnetic nanoparticles (NMP) together with anticancer or radiosensitizer drugs at the tumor site. We compared the iron-oxide particles binding characteristics with nitroimidazole in monoaxenic avirulent E.*histolytica* NIH 200, Candida *albicans* and Salmonella *BR 509* alongwith their membrane lipids and drug sensitivity. Major results were: 1. Microscopic experiments showed engulfed MNP particles passaged across the microbe membrane; 2.E.histolytica was oval shaped; Candida was thread like structure with capability of iron and manganese synthesis in situ; and Salmonella *BR 509* was rod shaped 1 micron in size; 3. The nano-sized particles were diffused slowly into the cell membrane by stimulation of colony stimulating factor; 4.The nitroimidazole coated nanoparticles embedded in microbe membranes showed sequential reduction of gluconeogenesis and energy metabolic integrity loss in microbes due to their interaction with host tissue sites; 5.The MNP-loaded micro-organism showed slow drug delivery rate from micro-organisms dependent on size of nanoparticle, composition of microorganism membrane; 6. The comparison showed the nitroimidazole release rate was dependent on medium pH, temperature, nutrients used and colony stimulating factor in Candida > Salmonella > E.histolytica at temperature range of 41.5to 42.5˚C and magnetic field at 0.5-1 MHz;7. At high magnetic field, the micro-organism cells showed necrosi s, loss of cell viability. In conclusion, the mechanism of nitroimidazole controlled release across microbial membrane depends on physiological pH, culture medium composition, nanoparticle size, colony stimulating factor, magnetic field and temperature. The mechanism of controlled drug delivery by avirulent micro-organisms has significance in designing a targeted anticancer drug therapy to focal tumors.

Key words: *drug delivery, tumor, nitroimidazole, magnetic nanoparticles, gluconeogenesis*

1 INTRODUCTION

2'nitroimidazole compounds and analogs are becoming state of art radiosensitizers and chemosensitizers in cancer prevention and management. Apart from their newly discovered antitumor properties 2' nitroimidazoles had been proven antiparasitic drugs. However, their toxicity was remained a major issue as they showed strong DNA breaking properties by direct DNA binding and coupling with DNA strands and it caused skepticism of nitroimidazole acceptability as safe choice of non-cancer therapeutic value. The binding of nitroimidazole with polymer depends on its hydrophilic bonds over its molecules as shown in Figure 1.

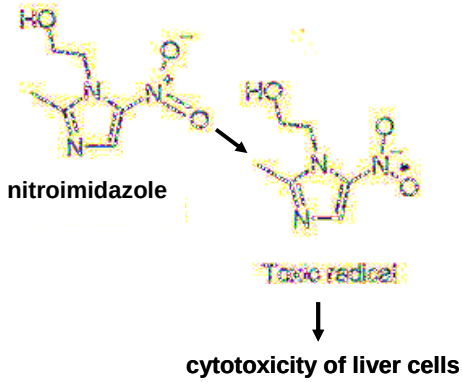


Figure 1: The structure of 2'nitroimidazole is shown with active hydrophilic groups to bind with polyethylene

2 NITROIMIDAZOLES AS RADIOSENSITIZERS AND ANTIPARASITIC DRUGS

The nitroimidazole is extensively in the following uses:

1. Nitroimidazole and its use in infections and tumor hypoxia, gluconeogenesis and energy metabolic integrity loss. Nitroimidazole serves as tumor sensitive drug. Its role is significant as blocker of

gluconeogenesis and metabolic integrity loss and glucose utility/metabolic energy blockers in tumors. It spares normal cells with normal flow of energy and oxygen in cell.

2. Salmonella coats: The drug bound MNP particles passaged across the microbe membrane. The membrane is made of lipoproteins and embedded with ion channels and ion sensitive permeability.

3. Candida coats: They have the capability of iron and manganese synthesis.

4. We established the value of single dose of nitroimidazole in treatment of Entamoeba histolytica.

5. Nanoparticle preparation and delivery: MNP-loaded micro-organism showed slow drug delivery based on physiological condition of the barrier. Available technology of MNP targeting by bacteria.

6. The effect of nitroimidazole dependent on temperature, nutrients used and of colony stimulating factor in liver cells.

7. At high magnetic field, the micro-organism cells showed necrosis, loss of cell viability.

Table 1: Use of nitroimidazole in therapy

Antibiotics	Antiparasitic	Hypoxia	Radiosensitizer
Mycobacteria	E.h, Gingiva	liver	liver
Candida	colon	colon	
Salmonella		breast	breast
Kleibsella		prostate	prostate
Shigella			

2.2 Other synthesis methods of polymer nitroimidazole complexes

2.2.1 Different polymers used for nitroimidazole

Chitosan hydrogel beads were prepared by the cross-linking method followed by enteric coating [1]. A C-P-A film consisting of a chitosan top layer and sodium alginate sublayer was synthesized and separated by an ornidazole(nitroimidazole)-incorporated poly(vinyl alcohol) layer [2]. The xanthan gum, pectin, carrageenan, beta-cyclodextrin (CD) or methacrylic acid-g-guar (MAA-g-GG) gum were used to coat nitroimidazole to design complexes [3]. The branched poly(ethylene glycol) of 5,000, 10,000 and 20,000 Daltons size were used for ester linkage between polymer and nitroimidazole to make complexes [4]. The calcium pectinate coating of nitroimidazole offered as ideal drug carrier [5].

2.2.2 The nitroimidazole polymer encaged inside bacteria carrier delivery system

In this direction progress is made slowly but promising results showed that nitroimidazole can target the tissue site very precisely. Several bacteria coats such as dead salmonella, gentamycin, helicobector pylori and amoeba microbes have been found as potential candidates as targeted nitroimidazole delivery. Interestingly, the path of nitroimidazole polymer complex can be visualized if it is designed to have magnetic particles in the center. We achieved results using iron-oxide nanoparticles coated with polyethylene and polystyrene for getting images by magnetic resonance imaging. Recently Haik et al 2008 reported a general strategy of designing salmonella coated magnetic nanoparticles to image temperature distribution in tumors in animals [6].

2.2.3 General strategy of preparing salmonella carriers: The cultured *Salmonella* strain BRD509 1 x 10^8 colony units if incubated with 30-100 micrometer sized iron-oxide particles for 120 minutes in saline buffer (3 times) leaves bacterial coats filled with iron oxide after magnetic separation and washing at 24ºC. These bacterial coats serve as MNP-loaded *Salmonella* organisms in tumor targeting in vivo. For preparing radiosensitizer nitroimidazole and polymer binding avidin or ferritin proteins are used. Previously we reported the design of iron oxide bound biotin-polymer complex and this scheme may be used for nitroimidazole. Benefits of salmonella carriers are: 1. they are immunocompatible in blood stream; 2. they act naturally but are avirulent; 3. due to no active genetic setup inside these bacteria donot interfere with tissue and immune defense system; 4. they get pass unrestricted across the membrane barriers with load of drug(nitroimidazole in our case) attached with superparamagnetic particles (iron-oxide or gadolinium) and become visible by imaging techniques; 5. they are not captured by macrophages due to their intact membrane phospholipids and enzyme composition; 6. they have capacity to live and combat against tumor cells like live virulent bacteria; 7. the bactaria coats provide slow drug release dependent on physiological conditions and do not change the drug properties. The unique superparamagnetic property of magnetic particles makes them visible by both MRI and computer tomography (CT) methods.

2.2.4 **The nitroimidazole release across polymer cage**

The method offered a cumulative percentage of the loaded drug that appeared in these serial supernatant fractions was plotted vs. time.

Mechanism of avirulent bacterial delivery across tumors: Kinetics of nitroimidazole-iron-oxide complex delivery and controlled release depends drug concentration across the membrane. The percentage of the drug that was loaded into each polymer and that was released vs. time was fit to the power function of the form $y = (a) \times tb$, where y is the cumulative released agent, a and b are constants and t is time (days).

Present study is preliminary report on comparison of three avirulent bacteria, E.histolytica coats as nitroimidazole-iron oxide complex delivery systems.

3 MATERIALS AND METHODS

3.1Cultures of bacteria and E.histolytica

NIH 200 cultures were maintained in aseptic monoaxenically as described elsewhere.

3.2 Nitroimidazole-iron oxide complex preparation

The iron-oxide maghemite was prepared by electrochemical method and incubated with biotin-polymer as previously described. The nitroimidazole 40 % solution was incubated with polymer complex to bind at 24 degree for 120 minutes. The bound complex was further mixed in cultured microbes. The microbes engulf the particles.

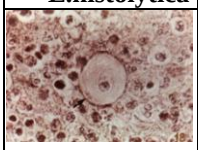
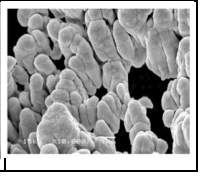
3.3 Evaluation of controlled release

For measurements of controlled nitroimidazole drug release, triplicate polymer discs were incubated for known intervals in 2 ml 0.1 phosphate-buffered saline, pH 7.4, 37 degrees C [23].

3 RESULTS

1. Microscopic experiments showed engulfed MNP particles passaged across the microbe membrane;
2. E.histolytica was oval shaped; Candida was thread like structure with capability of iron and manganese synthesis in situ; and Salmonella BR 509 was rod shaped 1 micron in size.

Table 1: The coating of nitroimidazole carriers are shown under microscope

E.histolytica	Candida	Salmonella
		

3. The nano-sized particles were diffused slowly into the cell membrane by stimulation of colony stimulating factor;
4.The nitroimidazole coated nanoparticles embedded in microbe membranes showed sequential reduction of gluconeogenesis and energy metabolic integrity loss in microbes due to their interaction with host tissue sites;
5.The MNP-loaded micro-organism showed slow drug delivery rate from micro-organisms dependent on size of nanoparticle, composition of microorganism membrane;
6. The comparison showed the nitroimidazole release rate was dependent on medium pH, temperature, nutrients used and colony stimulating factor in Candida > Salmonella > E.histolytica at temperature range of 41.5 to 42.5˚C and magnetic field at 0.5-1 MHz;

7. At high magnetic field, the micro-organism cells showed necrosis, loss of cell viability.

4 DISCUSSION

The nitroimidazole incubation in presence of microbes and magnetic particles showed the compatibility with additives.

- The end microbes showed the Nitroimidazole and its interaction in infections and tumor hypoxia, gluconeogenesis and energy metabolic integrity loss
- Salmonella: It served as carrier to MNP particles passaged across the microbe membrane
- Candida: showed capability of iron and manganese synthesis inside microbes while remaining avirulent
- Entamoeba histolytica as avirulent carrier
- Nanoparticle preparation and delivery: MNP-loaded micro-organism showed slow drug delivery
- Effect of temperature, nutrients used and colony stimulating factor
- High magnetic field exposure on the micro-organism cells showed necrosis, loss of cell viability.

The polymer bound nitroimidazoles compounds such as metronidazoles, etanidazoles, tinidazoles have been found useful as hypoxia markers in tumors [5, 18, 23]. Nitroimidazole polymer complexes as hydrogels, beads used as colon targeted drug delivery systems is emerging [1,3,7,8,9,10,12]. Different polymers such as polyethylene glycol [3], poly vinyl alcohol, polymethylmethacrylate[16],polysaccharides [3,11]. Other uses of nitroimidazole polymer complexes as delivery systems are reported in periodontal diseases [2, 18], amebiasis [3, 13, 19], helicobector pylori [7], gentamycin [13], vaginitis [5], gastritis [7, 9], sprays [16], liposomes [19].

The microbes have been reported as potential coating to carry and transport nitroimidazoles encaged in polymers. Of mention, gentamycin, helicobector pylori and amoeba microbes have been found as potential candidates as targeted nitroimidazole delivery[4, 13, 20]. Other options of liposomes as nitroimidazole delivery systems have been reported as minicapsular extrusion system [20].

The technique of using non-pathogenic bacteria such as Salmonella is an innovative approach in tumor as they replicate and exceed 1000-fold their concentration in tumor. *Salmonella typhimurium* strains accumulate at tumor sites and serve as carriers for drug bound contrast

agents. The bacterial delivery system may serve to transport with nitroimidazole or chemosensitive drugs to the tumor site. *Salmonella* BR 509 (rod shaped with 1 micron in length) incubated with *drug-bound polymer complex may be developed as tumor targeting systems.* Our nitroimidazole transport experiments suggested that small size of Mn^{+} particles were diffused into the Entamoeba histolytica cell membrane [21]. The liver cells (Kupffer cells) engulfed the iron oxide bound nitroimidazole in cultures and suggested that iron can function as contrast agent for imaging such as magnetic resonance imaging. The use of attenuated *Salmonella typhimurium* BRD509 in targeting of drug-coated MNP-loaded *Salmonella* organisms to tumor sites was reported in administration of the anti-cancer drug as it would significantly reduce the toxicity associated with prolonged treatment with high doses of chemotherapeutic drugs [22]. The dextrans dicarboxylic acid hemiester conjugates serve as potent prodrugs for nitroimidazole drug release in liver. The release kinetics was dependent on time [23].

ACKNOWLEDGEMENTS

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