Newer Methods of Nanoparticle Synthesis: Nitroimidazole properties with Nanometal oxides in Polymer Cages as Drug-Biomarker Monitors

R.Sharma*

*Center of nanomagnetics and Biotechnology, Florida State University,

Tallahassee, Florida 32310.Email: rksz2004@yahoo.com

ABSTRACT

Nitroimidazoles are radiosensitizers and hypoxia detecting chemosensitizers. Intially nitroimidazoles were single dose antibiotic drugs. Recently, nitroimidazole derivatives have emerged as multifunctional "drug-biomarker monitors" chemical compounds with importance in treatment of tumors, monitoring hypoxia and safer imaging contrast agents to monitor the therapeutic progress. Additionally, the number of nitroimidazole derivatives is growing and identified their importance as multifunctional nanoparticles. The nanoparticle synthesis scheme of nitroimidazole was proposed as nitroimidazole caged with metal oxide inside polymer coating and labeled with marker biomolecules. We report the step by step user-friendly new co-precipitation schemes of nitroimidazole carrier nanoparticles with their mechanisms of drug controlled release behavior, metal oxide sensitivity to imaging modalities and polymer coating chemistry developed at our lab. The multifunctional nitroimidazole nanoparticles were useful in detection and monitoring of hypoxia, cancer chemotherapy and soft tissue infections. In conclusion, nitroimidazoles are potential multifunctional molecules useful in chemotherapy, antiparasitic and monitoring hypoxia with greater possibility of simultaneous use of radiolabeled 2'nitroimidazoles as radiosensitizers, MRI-PET-US contrast imaging agents.

Key words: *nitroimidazole, radiosensitizers, imaging agent, antiparasitic, MRI-PET*

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.

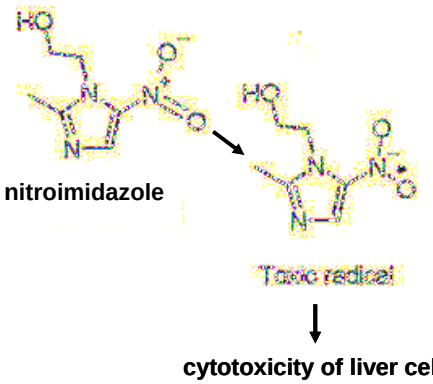

Figure 1: The structure of 2'nitroimidazole is shown with active hydrophilic groups to bind with polyethylene

2 SYNTHESIS OF NITROIMIDAZOLE POLYMER COMPLEXES

2.1 The synthesis of nitroimidazole polymer complexes

The coprecipitation methods were used in our center since the inception of innovative development at center of nanomagnetics and biotechnology using Bang's manual initially and over years the technology was modified for different nanoparticles for various purposes [1]. Mainly the coprecipitation method includes the general steps of: 1. A batch process to develop and prepare the composite particles. A 0.05% w/w; 10 ml solution of polyethylene wax (number average MW of 700 g/mole (Honeywell Corp.) using decaline or octamethylcyclotetrasiloxane OMCTS (Dow

Chemical Company) at 150°C; 2. To this solution, iron oxide powder is added with 30%-50% w/w polyethylene, and sonicated at 50% amplitude for 30 seconds; 3. Then, 10 ml of tetraglyme ("TG") (obtained from Sigma-Aldrich) is added to the iron-oxide polyethylene mixture at 150°C, and sonicated at 50% amplitude for about 30 seconds; 4.Next, the mixture is immediately cooled to about 0°C in ice water held at 0°C. Within three to four minutes, the polyethylene-iron-oxide mixture is transformed into an emulsion with microdroplets made of supercooled polyethylene wax solution and iron oxide dispersed in a continuous phase of nonsolvent; 5. The emulsion is warmed to room temperature 27°C for 45 minutes to make polyethylene and maghemite particles suspended in the emulsion. This emulsion is cooled to -10°C for half an hour to form a macrophase separated system made of thin reddish-brown sandwiched between top solvent layer and non-solvent bottom layer; 6. The reddish-brown layer of polyethylene/iron oxide particles is centrifuged to isolate the particles from solvent mixture. This general strategy was used at our center over several years as described elsewhere [1].

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 [3]. 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 [4]. 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 [6]. 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 [9]. The calcium pectinate coating of nitroimidazole offered as ideal drug carrier [10].

2.2.2 The nitroimidazole release across polymer cage

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

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).

3 DISCUSSION

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 [1,3,6,11,13,14,15,17,22]. Different polymers such as polyethylene glycol [9,20], poly vinyl alcohol [4,24], polymethylmethacrylate[16],polysaccharides[6,11]. Other uses of nitroimidazole polymer complexes as delivery systems are reported in periodontal diseases [2, 21], amebiasis [6, 13, 22], helicobector pylori [7], gentamycin [16], vaginitis [8], gastritis [10, 12], sprays [19], liposomes [25].

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[7, 16, 22]. Other options of liposomes as nitroimidazole delivery systems have been reported as minicapsular extrusion system [25].

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 [26]. 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 [27]. The dextrans dicarboxylic acid hemiester conjugates serve as potent prodrugs for nitroimidazole drug release in liver. The release kinetics was dependent on time [28].

The nitroimidazole radiosensitizers as magnetic nanoparticles are emerging as potents tools in hyperthermia and hypoxia monitoring of tumor at different locations. The double lebeling techniques utilize their multifunctionality and new techniques are likely to evolve for nitroimidazoles as therapeutic and imaging contrast complex simultaneously for using them in MRI-CT-PET multimodal imaging [29]. In this direction, some progress is made on double labeling by 18,19-F nitroimidazole compounds (FITNIM), 131,132-I and 99Tl labeled nitroimidazole compounds in SPECT use. Newer nitroimidazole derived compounds are coming up for computed tomography and simultaneous hyperthermis therapy while nitroimidazoles may serve both as targeted drug and imaging contrast. Etanidazole, piconidazole have been successfully marketed for hypoxia biomarker imaging agents. Metronidazole derivatives still remain as old gold choice in targeted delivery in colon infections. In future, nitroimidazoles will have a bright choice of cell microimaging by fluorescent labeling and tracking the intracellular mitochondrial and lysosomal changes. It can not be said if nitroimidazoles may serve as targeting intracellular DNA. However, its derivatives and analogs have been reported as DNA strand breaking agents. Apart form this fact, nitroimidazoles exhibit cytotoxicity and this property may be proven as biomarker of cytotoxicity inside cell. It remains to see more on cytotoxicity is not severe and within biocompatibility limits.

CONCLUSION

The nitroimidazoles are potent radiosensitizers, and are well accepted antiparasitic compounds. Their slow release across colon and tumor is a challenge due to their cytotoxicity caused by their DNA binding properties.

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