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PRINCIPAL INVESTIGATOR: Bahar Zarabi
Hamid Ghandehari, Ph.D.

CONTRACTING ORGANIZATION: University of Maryland Baltimore
Baltimore, MD 21201

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Hamid Ghandehari, Ph.D.

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14. ABSTRACT

The overall purpose of this research is to develop a polymeric drug delivery system containing magnetic resonance contrast agents for the treatment of breast cancer. This drug-imaging agent delivery system will allow the follow up of the fate of the drug delivery system and its relation to reduced tumor mass, improved efficacy and reduced toxicity in individual patients. In year one progress was made in the following areas: 1) Synthesis of polymer-linked contrast agent conjugates; 2) Physicochemical characterization of polymer-linked contrast agent conjugates; 3) Relaxivity and stability measurements of polymer-linked contrast agent conjugates. In addition I completed my coursework (overall GPA=3.6), presented the results in the 2005 Era of Hope Meeting, and participated in other scientific meetings related to my field.

15. SUBJECT TERMS
Polymers, drug delivery, breast cancer, contrast agent, MRI

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INRODUCTION

The **long term objective** of this research is to develop a polymeric drug delivery system containing magnetic resonance contrast agents for treatment of breast cancer. This drug-imaging agent delivery system will allow the follow up of the fate of the drug delivery system and its relation to reduced tumor mass, improved efficacy and reduced toxicity in individual patients. Two specific aims were proposed:

1) To synthesize a series of polymer-drug-imaging agent conjugates.

2) To characterize the conjugates by physicochemical methods.

In year one of this project, progress was made to partially accomplish Aims 1 & 2, i.e., we synthesized and characterized the polymer-nitroxide and polymer-dinitroxide conjugates without the drug. In doing so Tasks 1, 2, and 3 of year one and Task 1 of year two of Statement of Work were accomplished. In addition related areas were explored as outlined in the body of this report.

BODY

A. Synthesis of the proposed comonomers and copolymers

For this portion of the project the following tasks were proposed:

1. Synthesis of comonomers
2. Synthesis of copolymers

We made progress in items 1 and 2 and disseminated the results in a poster presentation at the Era of Hope (2005) conference (Please see Appendix 1). Contrary to what we expected attachment of dinotroxides did not result in increased relaxivity. As a consequence we explored the possibility of synthesizing gadolinium – containing copolymers. Therefore in addition to what was proposed we also synthesized polymer-linked gadolinium conjugates (Please see Appendix 2).

B. Characterization of the copolymers

In year one, the following tasks were proposed for this part of the project:

1. Physicochemical characterization of the comonomers

2. Physicochemical characterization of the copolymers

3. Relaxivity measurements

4. Stability studies

We were able to accomplish tasks 1 to 4 and the results are reported in Appendix 1 (Results and Discussions). Since the conversion rate of dinitroxide was low (approximately 50%) the relaxivity of these conjugates were not as expected. Then based on different synthetic methods we increased the conversion rate of dinitroxide to about 80% but we did not get higher relaxivity compared to nitroxide conjugates. Therefore in addition to what was proposed we synthesized and characterized a polymer-linked gadolinium conjugate (Appendix 2).

KEY RESEARCH ACCOMPLISHMENTS

1) Synthesis and characterization of polymer-linked nitroxide, polymer-linked dinitroxide and polymer-linked gadolinium conjugates.

2) Compared the relaxivity of polymeric contrast agents with free contrast agents.

3) Compared the stability of polymeric contrast agents with free contrast agents.

4) Presented this research in the Era of Hope Meeting.

5) Also participated in scientific meetings related to my area of study, namely controlled drug delivery: American Association of Pharmaceutical Sciences (AAPS-November 2004, Baltimore, Maryland) and Controlled Release Society (CRS-June 2005, Miami, Florida) meetings.

6) Accomplished my course work (current GPA :3.6)
-Aspects of solid dosage forms (A)
-Pharmacometrics/ experimental design (A)
-Mechanism of organic reactions (B)

7) Started preparation for my qualifying examination to be held in Sep. 2005

8) Was initiated as an active member of the Rho Chi Society (Pharmaceutical honor society) based on my performance in school.

REPORTABLE OUTCOMES

1) Poster presentation in Era of Hope (2005)

2) Departmental seminar

3) Completion of course work and preparation for comprehensive examinations

CONCLUSIONS

In summary progress was made in the following areas:

1) Synthesis of polymer-linked contrast agent conjugates

2) Physicochemical characterization of polymer-linked contrast agent conjugates

3) Relaxivity measurement of polymer-linked contrast agent conjugates

4) Stability mesurments of polymer-linked contrast agent conjugates

Due to the lower relaxivity of polymer-linked dinitroxide conjugates than polymer-linked nitroxide conjugates (probably due to structural changes in dinitroxide) and successful synthesis and characterization of polymer-linked gadolinium we intend to continue this project based on gadolinium contrast agent.

REFERENCES

None

APPENDIXES

Appendix 1: Bahar Zarabi, Jiachen Zhuo, John Weaver, Gerald Rosen, Rao Gullapalli, and Hamid Ghandehari. Synthesis and characterization of a novel macromolecular magnetic resonance imaging contrast agent. Poster Presentation at the 4th Era of Hope Meeting, Philadelphia, PA, June 8-11, 2005.

Appendix 2: Chemical structure and characteristics of polymer-linked gadolinium conjugate.

APPENDIX 1: B. Zarabi, J. Zhuo, J. Weaver, G. Rosen, R. Gullapalli, and H. Ghandehari, Synthesis and Characterization of a Novel Macromolecular Magnetic Resonance Imaging Contrast Agent, Poster Presentation at the 4th Era of Hope Meeting, Philadelphia, PA, June 8-11, 2005.

OBJECTIVE

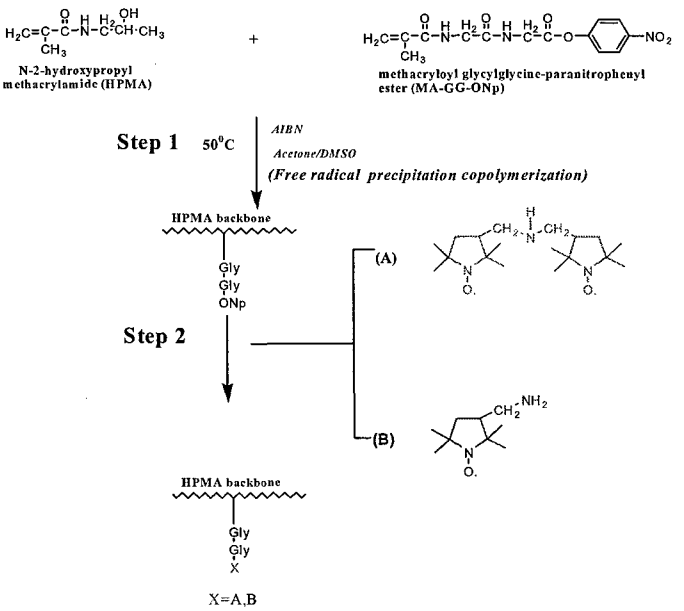
To synthesize and characterize novel N-(2-hydroxypropyl) methacrylamide (HPMA) copolymer-nitroxide conjugates with improved relaxivity for Magnetic Resonance Imaging (MRI) of breast cancer solid tumors.

INTRODUCTION

Early detection of tumors and characterization of their response to therapy are fundamental challenges in the quest to improve cancer survival rates (1). Dynamic magnetic resonance imaging is often used for diagnosis and prognosis of solid tumors. The most commonly used magnetic resonance contrast agents (CAs) are gadolinium chelates. However, these agents are not tissue selective and generally have a short residence time in tumor. Recent investigations have demonstrated that macromolecular CAs have longer lifetime in the blood pool and higher accumulation in solid tumors in comparison to low molecular weight CAs. N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers are one class of polymeric carriers that show promise for targeted delivery of drugs and imaging agents. These polymers are non-immunogenic and can be tailored to the characteristics of the specific target (2). We previously investigated the potential of HPMA copolymer-mononitroxide conjugates as MRI contrast agent (3). Challenges for the use of nitroxide based contrast agents in MRI are their low magnetic relaxivities and high bioreduction rate. Compounds that contain multiple nitroxides have been shown to have higher relaxivities and stabilities (4). The objective of this research was to attach dinitroxides to HPMA copolymers and evaluate their relaxivity and bioreduction.

EXPERIMENTAL METHODS

SYNTHESIS OF POLYMER-IMAGING AGENT CONJUGATES (SCHEME 1)



Synthesis of HPMA copolymer - contrast agent conjugates: First, HPMA copolymeric precursors containing side chains terminated in 5, 15, and 30 mole% of p-nitrophenyl ester (ONp) were synthesized by free radical precipitation copolymerization using AIBN as the initiator (Scheme 1). In the second step, a mononitroxide, namely 3- (aminomethyl) 2,2,5,5-tetramehyl-1-pyrrolidinoxyl, and dicyclic analogs of this compound terminated in secondary amine (dintroxide) were attached to the polymeric precursors by an aminolysis reaction.

Characterization of conjugates: HPMA copolymer p-nitrophenyl ester (ONp) were characterized for the contents of ONp spectrophotometrically (λmax=400) and molecular weight and molecular weight distribution by size exclusion chromatography (Superose 12 HR 10/30 column, Pharmacia, NJ) (Table1). The conjugates were characterized for nitroxide and dinitroxide content using UV spectrophotometry (λmax=260).

PHYSICOCHEMICAL CHARACTERIZATION (Table 1)

sample	HPMA (mole%)	MA-G-G-ONP (mole%)	ONP content (mmole/g polymer)	Mw (g/mole)	n[a]
P1	70	30	1.41±.037	22000	1.5
P2	85	15	0.696±.012	41000	1.8
P3	95	5	0.273±.005	37000	1.3

[a] Polydispersity

Relaxivity measurements: The T1 relaxivity (r1) of HPMA copolymer-linked nitroxides and dinitroxides were calculated at room temperature and 1.5 tesla (T). The relaxivity of mononitroxide, dinitroxide, and gadolinium diethylenetriaminepentaacetic acid (Gd-DTPA) were also measured as controls (Table 2).

Stability test: The reduction of HPMA copolymer-nitroxide and –dinitroxide (50 μM) conjugates in the presence of glutathione (1mM) was evaluated by Electron Paramagnetic Resonance (EPR) spectroscopy (E-109,Varian Associates, CA) at room temperature (Figure1).

RESULTS AND DISCUSSIONS

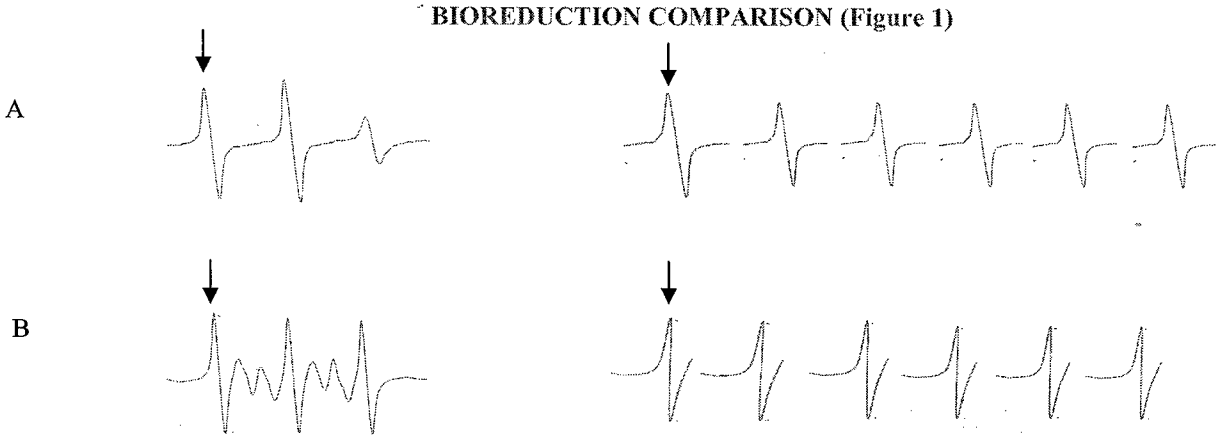
Relaxivity of conjugated nitroxides and dinitroxides when attached to polymers increased compared to free nitroxide and dinitroxide (Table 2). These macromolecules are more efficient with respect to their relaxivity as a consequence of their increased rotational correlation time resulting from their high molecular weight and higher nitroxide content. The relaxivity of polymeric conjugates of nitroxides and dinitroxides exceeded that of standard CA Gd-DTPA. Results suggest that the relaxivity of HPMA copolymer-dinitroxide conjugates with 50 mole% conversion for P2 and P3 is less than and for P1 equal to r1 relaxivity of HPMA copolymer-mononitroxide conjugates with 100 mole% conversion. The reasons for this anomalous behavior are subject of further investigation. In vitro stability test in the presence of glutathione showed that the polymeric conjugates and selected structures of free dinitroxide and mononitroxide are relatively stable (Figure 1).

CHARACTERIZATION OF POLYMER-LINKED NITROXIDES (Table 2)

SampleNo.	Sample Description	Nitroxide Content (mmole/gpolymer)		Relaxivityb (mM-1.s-1)
		Nitroxide	Dinitroxide (2°)a	
1	P1-Nox	1.43±0.004	-	10.38
2	P1-Dinox (2°)	-	0.764±0.054	10.58
3	P2-Nox	0.704±0.019	-	11.83
4	P2-Dinox (2°)	-	0.375±0.021	8.61
5	P3-Nox	0.278±0.005	-	4.24
6	P3-Dinox (2°)	-	0.136±0.005	2.86
7	Nitroxide	-	-	0.26
8	Dinitroxide (2°)	-	-	0.52
9	Gd-DTPA	-	-	5.2

[a] Secondary amine dinitroxide
[b] Data for relaxivity are the average of duplicate experiments

BIOREDUCTION COMPARISON (Figure 1)



A) 1: Sample 8 EPR; 2: Sample 8 EPR at different time points in the presence of glutathione.
B) 1: Sample 6 EPR; 2: Sample 6 EPR at different time points in the presence of glutathione.

CONCLUSIONS

- Compared to free nitroxides HPMA copolymer conjugated nitroxides demonstrated higher relaxivities than Gd-DTPA.
- Selected free and polymeric conjugated nitroxides show higher stabilities than previously synthesized free nitroxides (5).
- These studies demonstrate the potential of HPMA copolymer-nitroxide conjugates for imaging of breast cancer tumors.

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ACKNOWLEDGEMENTS

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APPENDIX 2: Chemical structure and characteristics of polymer-linked gadolinium conjugate.

Sample	APMA Content (mmol/g polymer	DOTA content (mmol/g polymer)	Gd content (mmol/g polymer)	M_w (g/mole)	Polydispersity
HPMA-10%APMA	0.47	0.44	0.38	27000	1.4