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Award Number: W81XWH-05-1-0434

TITLE: A Polyamine Oxidizing Enzyme as a Drug to Treat Breast Cancer

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REPORT DATE: July 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-07-2006		2. REPORT TYPE Annual		3. DATES COVERED (From - To) 20 Jun 2005 - 19 Jun 200	
4. TITLE AND SUBTITLE A Polyamine Oxidizing Enzyme as a Drug to Treat Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-05-1-0434	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) William S. McIntire, Ph.D. E-Mail: william.mcintire@ucsf.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Northern California Institute for Research & Education (NCIRE) San Francisco, CA 94121				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT: The purpose of the research is to test modified forms of bovine serum amine oxidase (SAO) as effective treatments for breast cancer, using a mouse model. If successful, this approach, or a variation thereof, may eventually be used as a therapy for breast and other cancers in humans. Currently, a large quantity of very pure bovine PAO is in hand, which was obtained from 10 gallons of fresh cow blood. A method has been developed for generating extremely pure enzyme, which will be deglycosylated before being polyethylene glycolated (PEGylated). PEGylated SAO will be tested for toxicity and for its ability to slow the growth or shrink the size of breast tumors implanted in test mice. PEGylated SAO should target tumors but have little effect on normal tissue. Once concentrated in a tumor, the active PEGylated SAO will oxidize acetylated polyamines, which are excreted by tumor cells in large quantities. When the acetylated polyamines are oxidized, cytotoxins are generated.					
15. SUBJECT TERMS cancer, tumor, breast cancer treatment, polyamines, serum amine oxidase, plasma amine oxidase, drug design					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	6	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover.....	
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	5
References.....	5
Appendices.....	6

ANNUAL PROGRESS REPORT (2006)
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INTRODUCTION

We want to target breast tumors with high levels of an enzyme that oxidizes efficiently N^1 -acetyl-spermine and N^1 -acetyl-spermidine. These acetylated polyamines are exported from tumor cells at high levels. We hypothesize that toxic oxidation products will be generated locally in sufficient quantities to slow or arrest the growth of the tumor cells, or kill these cell, without harming substantially non-cancerous tissues (1, 2). For this work, we have chosen the enzyme bovine serum amine oxidase (SAO), which can be obtained in large quantities in a very pure form (3).

The proposed work can be considered as nanotechnological in nature since each enzyme molecule of SAO is polyethylene glycol(PEG)-encapsulated. This allows the enzyme to target tumors with high specificity; due to its high vascularization and unusual nature of the capillaries surrounding a tumor, an intravenously injected PEGylated enzyme will target specifically malignancies, but not normal tissues (1, 2, 4). The PEG-coated enzyme has enhanced stability, is protected from proteolysis, and is not antigenic. These properties afford the PEG-enzyme an increased lifetime, and, hence, an increased circulation time relative to the unmodified form (1, 2). The goal of the research is to inject deglycosylated and PEGylated SAO into the blood stream of tumor-bearing mice to determine if this treatment is a viable anticancer therapy.

BODY

TASK 1. Prepare two polyethylene glycol(PEG)-derivatives of deglycosylated bovine serum amine oxidase (SAO).

It required considerably more time than anticipated to obtain the required large amount of pure bovine SAO. With the hope of saving time and resources, we attempted to purify this enzyme from a crude commercial preparation (product # LS003114, Worthington Biochemical Corporation). However, after a prolonged effort, we obtained about 1 mg of a fairly pure SAO from about 30 mg of the crude material, far too little to be of any use.

In order to obtain the requisite amount of SAO, we procured 10 gallons of fresh cow blood from a local slaughterhouse. By following a published procedure, with little modification, we obtained highly pure SAO (3). It required about two weeks of set up for the purification and about 2 months to get about 2 grams of the enzyme.

While very pure, the SAO still had low levels of contaminants that could interfere with its deglycosylation and PEGylation, and possibly obscure the outcome of experiments to test the treatment as an anticancer therapy. Hence, another few weeks were expended to identify a final step to remove the contaminants. We found that chromatography on a Macro Prep Type I Ceramic Hydroxyapatite (Bio-RAD) column work very well for this purpose (5).

We predict that it will require another few weeks to obtain enough extremely pure enzyme for the remainder of our studies. After the purification is complete, we will run trial experiments to identify conditions for the efficient deglycosylation of bovine SAO. We will begin by following a published procedure (6). Deglycosylation is necessary in order to eliminate any

interference with the function of the mouse's own blood-borne SAO. Once deglycosylated bovine SAO is in hand, the enzyme will be PEGylated as describe in the literature (1, 2).

TASK 2. Test the general toxicity of the two PEG-SAO derivatives.

This task cannot be initiated until we prepare a large amount of deglycosylated and PEGylated forms of bovine SAO (see **TASK 1**).

TASK 3. Test each PEG-SAO conjugate as an antitumor agent using mice with implanted human tumors.

This task cannot be initiated until we prepare a large amount of deglycosylated and PEGylated forms of bovine SAO (see **TASK 1**).

KEY RESEARCH ACCOMPLISHMENTS

- Procurement of a large quantity of extremely pure bovine PAO for deglycosylation and PEGylation.

REPORTABLE OUTCOMES

Currently, the only reportable outcome is that we have obtained the requisite amount of pure bovine PAO for the remainder of our research on this project.

CONCLUSION

Since we have not yet done any animal work, we cannot report any conclusions. If our hypothesis is correct, the treatment may one day be an effective anticancer therapy in human patients.

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APPENDICE

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