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PRINCIPAL INVESTIGATOR: William S. McIntire, Ph.D.

CONTRACTING ORGANIZATION: Northern California Institute for Research & Education
San Francisco, CA 94121

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14. ABSTRACT The research is aimed at testing two polyethylene glycolated (PEGylated) forms of bovine serum amine oxidase (SAO) as effective treatments for breast cancer using a mouse model. Hopefully, this approach, or a variation thereof, can be used as a new a therapy for breast and other cancers in humans. Currently, a large quantity of pure bovine PAO is available, which was obtained from 10 gallons of fresh cow blood. A final purification step has been used to produce large quantities of extremely pure SAO. Attempts to find a cost-effective, practical method for the deglycosylation of SAO were unsuccessful. Using several different reagents, we have found two that will be used to PEGylate SAO. Before testing as an anti-tumor agents, both PEGylated SAO derivatives will be tested for toxicity by using nontumorigenic mice. Once it is established that these agents do not have significant side effects, they will be tested for the 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 extracellularly in a tumor, 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.					
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INTRODUCTION

The purpose of the work is to target specifically breast tumors with high levels of an enzyme that oxidizes efficiently N^1 -acetyl-spermine and N^1 -acetyl-spermidine, which are exported from tumor cells at high levels. Presumably, the toxic oxidation products will be generated locally in sufficient quantities to slow or arrest the growth or kill tumor cells without harming substantially non-cancerous tissues (1, 2). For this work, we have chosen bovine serum amine oxidase (SAO), which has been obtained in large quantities in a very pure form from cow blood (3). The work can be considered as nanotechnological in nature since each molecule of SAO is polyethylene glycol(PEG)-encapsulated. This allows the modified 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 PEGylated SAO into the blood stream of breast tumor-bearing mice to determine if this treatment is a viable anticancer therapy.

BODY

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

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 protocol with little modification, we obtained pure SAO (3). While very pure, the SAO still had low levels of contaminants that could possibly interfere with its PEGylation and/or 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). This method was used to prepare enough enzyme for the initial phase of the work, i.e., to determine the toxicity of two PEG-SAO derivatives in non-tumorigenic mice (**TASK 2**).

Initially, we proposed to deglycosylate SAO before attempting PEGylation of the enzyme. It was thought that deglycosylation would reduce or eliminate any interference with the animal's serum SAO, or the mimicking of this endogenous enzyme. However, it was deemed to be prohibitively expensive to deglycosylate the required amounts of SAO for our experiments; it would cost tens of thousands of dollar to accomplish this goal. This unsuccessful endeavor required about 6 weeks of exploratory research. Next, we proceeded with the PEGylation of the fully native bovine SAO with the hope that the large PEG groups would partially or fully mask the

attached polysaccharide, thus, minimize interference with or mimicking of the mouse's own SAO.

A small amount of bovine SAO was PEGylated at pH 7.4 with Sunbright® ME-050CS (PEG MW = 5,000 Da) and Sunbright® ME-200CS (PEG MW = 20,000 Da) (NOS Corp., Tokyo, Japan). In addition, this enzyme was PEGylated at pH 8.0 with mPEG-SC MW 5,000 and mPEG-SC MW 20,000 (Laysan Bio Inc., Arab, AL). It was found that the Sunbright® reagents gave good PEGylation levels while retaining high enzymic activity. Hence, we will proceed with preparing larger amounts of two PEG-SAO derivatives using these reagents that are needed for **TASK 2**.

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

We predict that this work will begin in August or September, 2007, once the requisite amounts of the two PEG-SAO derivatives will be available (see **TASK 1**). This work will require injecting several non-tumorigenic mice with one PEG-SAO derivative, and injecting another group of mice with the second derivative.

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 the two PEGylated forms or bovine SAO (**TASK 1**), and we have tested these in nontumorigenic mice (**TASK 2**).

TASK 4. Test each PEG-SAO conjugate as an antitumor agent using mice with implanted with another type of human breast tumors.

This task cannot be initiated until we prepare a large amount of deglycosylated and PEGylated forms or bovine SAO (**TASK 1**), and we have tested these in nontumorigenic mice (**TASK 2**). This work will be started once **TASK 3** has been completed.

KEY RESEARCH ACCOMPLISHMENTS

- Procured a large quantity of extremely pure bovine PAO for deglycosylation and PEGylation.
- Attempted to efficiently and cost-effectively deglycosylate bovine SAO. After about 6 weeks of work, this endeavor was deemed untenable.
- Developed methods to produce two PEGylated derivatives of bovine SAO; one form of SAO will have about five 5,000 MW PEG groups attached, and the other form of SAO about five 20,000 MW PEG groups attached.
- Work is in progress to scale-up the production these two derivatives.

REPORTABLE OUTCOMES

The only reportable outcome is that we have obtained the requisite amount of pure bovine SAO for the remainder of our research on this project, and that we have developed methods to PEGylated the enzyme.

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

NONE

SUPPORTING DATA

NONE