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PRINCIPAL INVESTIGATOR: Richard Fisher, M.D.

CONTRACTING ORGANIZATION: University of Rochester
Rochester, NY 14642

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14. ABSTRACT This grant program encompasses two complimentary projects. The hypothesis that leukemia can be treated effectively by inhibition of putative cancer stem cells will be tested in project #1. This will be done by application of inhibitors of stem cells as a novel approach for eradication of leukemia tumor cells. Parthenolide (PTL)-based drugs and related drugs that inhibit nuclear factor kappa B (NF-κB) will be used. The effects of these drugs will also be tested on normal hematopoietic cells. In project 2, studies will investigate how standard therapies effect normal CNS stem cells, and will attempt to develop less toxic regimens for the treatment of brain cancers. To this end, studies will determine whether parthenolide or related drugs cause CNS damage in animals treated with these substances, and will assess whether parthenolide can function as a chemosensitizing agent for various conventional chemotherapy drugs.					
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Introduction

This grant is comprised of two complementary projects. For the purposes of this report, progress for each project will be described separately below.

Please note: Although this grant formally began on September 1, 2007, the work was not approved to begin until late February of 2008. This 6-month delay was due to logistical problems in obtaining approval for the use of experimental animals. All projects have now begun and are proceeding as planned, however, we request that progress be viewed with this delay in mind.

Body

Project 1

The objective of this project is to develop a novel therapeutic agent that specifically targets human leukemia stem cells (LSC). While the concept of a critical leukemia stem cell in myeloid disease has been postulated for over three decades, to date no therapeutic agent has been identified that specifically and preferentially ablates LSC in vivo. Thus, the central premise of this grant is that direct targeting of LSC will yield more effective therapy for leukemia. Previously, we demonstrated that parthenolide (PTL) is highly cytotoxic to LSC in vitro, but does not significantly affect normal hematopoietic stem cells (HSC). However, solubility of PTL is limiting; thus we have generated a PTL analog, dimethyl amino parthenolide (DMAPT), that is much more soluble in water and retains the anti-leukemic activity of PTL. Using this agent, the tasks below were specified:

SOW task #1: To demonstrate that a parthenolide analog can be used for preclinical and clinical applications related to treatment of chronic leukemia (Months 1-36).

Progress: we have tested DMAPT against a panel of primary chronic lymphocytic leukemias (CLL) and shown that the compound has significant activity. The LD50 was 3.0 micromolar, in good agreement with the studies of Hewamana et al (Blood, 2008, 111:4681). Between 3.0 and 5.0 micromolar DMAPT, depending on the particular specimen, we observe strong inhibition of NF- κ B with 2 hours, as determined by electrophoretic mobility shift assay. Further, we observe a strong induction of oxidative stress, as measured by labeling with MBBR, which detects loss of free thiol groups. Notably, pretreatment with reducing agents such as N-acetylcysteine completely abrogates the toxicity of DMAPT. This observation indicates that the oxidative stress created by the drug's activity is important for the biological activity observed.

SOW task #2: To demonstrate that a parthenolide analog can function as a chemosensitizing agent to enhance ablation of chronic leukemia cells (Months 37-60).

Progress: Not started yet

Project 2

The primary goal of this project is to investigate how standard therapies effect normal CNS stem cells and to develop less toxic regimens for the treatment of brain cancers:

SOW task #1: To determine whether parthenolide or parthenolide analogs cause CNS damage in animals treated with these substances, and to determine whether parthenolide or parthenolide analogs enhance the damage caused by cytarabine. (Months 1-24)

Progress: We have analyzed parthenolide for its toxicity on cells of the CNS and have found that while neuroepithelial stem cells are relatively resistant to the activity of this agent, the progenitor cells that make oligodendrocytes appear to be as vulnerable as are cancer cells. In order to determine the reasons for this we have analyzed the effects of the parthenolide on signaling pathway function in oligodendrocyte progenitor cells and have found that parthenolide suppresses pathway function that would not be predicted from a substance that simply inhibited NF-kappaB signaling. Instead, it appears that in these progenitor cells at least, parthenolide also has effects that indicate a broader suppression of receptor tyrosine kinase signaling.

It is possible, however, that the fact that parthenolide and its derivatives are water soluble will mean that toxic effects on the central nervous system in vivo will only be seen when the blood brain barrier is open. We will take delivery, in the next several weeks, of new microscopic equipment that will greatly speed up the time course of analysis of in vivo effects of chemotherapeutic agents. Once this equipment has arrived we will test the hypothesis that parthenolide is safe for the CNS but only when used in conjunction with agents that do not open the blood brain barrier.

SOW task #2: Demonstrate that mice in which purified cells are more oxidized in vitro will exhibit more extensive damage from cytarabine, parthenolide (or parthenolide analogs) or the combination of these agents, than those in which purified cells are intrinsically more reduced (Months 25-48).

Progress: Not started yet.

SOW task #3: To initiate identification of potential prognostic indicators to detect individuals at greater risk for adverse side effects of therapy with

cytarabine, parthenolide (or parthenolide analogs) or the combination of these agents, and begin testing to provide proof of principle for protective strategies that involve administration of N-acetyl-L-cysteine (alone or in combination with Vitamins E and/or C) as an anti-oxidant to protect against oxidative damage (Months 49-60).

Progress: Not started yet.

Key Research Accomplishments

Analysis of parthenolide with respect to toxicity towards leukemia cells and neuronal cell populations.

Reportable outcomes

Pending

Conclusions

Parthenolide-based drugs appear promising as agents for treatment of CLL, but it may be necessary to monitor the relative benefits of the drug with respect to oligodendrocytes.

References

N/A

Appendices

N/A