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PRINCIPAL INVESTIGATOR: Barbara Christy

CONTRACTING ORGANIZATION: University of Texas Health Science Center
San Antonio, TX 78229

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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of the proposed research is to determine if treatment with rapamycin (an FDA-approved drug that is well tolerated by patients) can prevent or improve the cognitive problems caused by chemotherapy treatments. To accomplish this goal, the first task proposed was to test different chemotherapy drugs in mice for their ability to produce cognitive defects in behavioral tests. We have tested three common chemotherapy drugs (5-Fluoruracil, Doxorubicin and Cyclophosphamide) using several behavioral tests (Passive Avoidance, Tail Flick and Novel Object Recognition). We have not yet demonstrated a significant cognitive effect for any of the drugs tested compared with control mice injected with saline. We are continuing to test higher doses of these drugs and are awaiting approval for the use of additional drugs. The second task proposed was to test the ability of dietary rapamycin to improve cognitive defects, using the drug or drugs that produced a measurable cognitive response. The performance of experiments relating to this task cannot begin until task 1 has been accomplished and the mouse model system established.					
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INTRODUCTION

Although advances in treatment have increased survival for breast cancer and other common cancers, many challenges remain. Although life-saving, chemotherapy treatments can be very harsh, and unpleasant side effects are common. Some of these side effects are temporary and well-tolerated by patients, while others may be lasting and negatively impact the quality of life during and after treatment. One commonly reported side effect of chemotherapy in breast cancer patients is “chemobrain” or “chemofog”, terms used to describe cognitive problems caused by treatment. These cognitive problems can be mild or severe, and may be long-lasting or even permanent in some patients. Problems in thinking, concentration, learning and memory are generally very distressing to patients and survivors and dramatically affect their quality of life. Finding drugs or other treatments which can prevent or alleviate chemotherapy-induced cognitive problems is an important goal which will dramatically impact the lives of cancer patients.

Rapamycin is an FDA-approved drug currently used in transplant patients for immunosuppression. Rapamycin and related drugs work by partially inhibiting the activity of the mTOR protein kinase, which regulates virtually every cellular metabolic process. Rapamycin is relatively safe and well-tolerated in human patients. Rapamycin has been shown to have beneficial cognitive effects in a mouse model of Alzheimer’s disease and in non-mutant mice as well (Spillman et al, 2010). Therefore, we proposed that rapamycin treatment may help prevent or improve cognitive problems caused by chemotherapy treatments.

In this project, we proposed to develop a mouse model for chemotherapy-induced cognitive problems. Problems in cognitive function will be assessed by behavioral testing. Once the mouse model system is established, we will test the ability of long-term dietary rapamycin to prevent or improve the cognitive problems following chemotherapy drug administration.

BODY

We proposed to address the problem in two aims. In the first aim, we proposed to establish a mouse model system for cognitive effects of chemotherapy. Several common chemotherapy drugs (5-Fluorouracil, Doxorubicin and Cyclophosphamide) have been tested, using dosages shown previously to produce changes in neurogenesis in the dentate gyrus (Janelins, M. et al, 2010). Thus far, we have not yet produced cognitive defects dramatic enough to be detected using behavioral testing. For behavioral testing, we have worked with a collaborator, Dr. Kathleen Fischer of the Barshop Institute for Longevity and Aging Studies at the University of Texas Health Science Center at San Antonio. Dr. Fischer is experienced in many different behavioral tests in mice and rats, and is currently involved in testing cognitive and physical parameters during aging.

In our first set of experiments (group A), 20 mice were used for the initial testing using Passive Avoidance and Novel Object Recognition. A tail-flick test was also used to assess anxiety. Mice were injected intraperitoneally 3 times over a one week period with either saline, 5-fluorouracil (60 mg/kg), or Doxorubicin (5 mg/kg). Mice were observed daily for signs of illness or impairment; no signs of impairment were noted. Mice were

weighed prior to the start of injections and following the last injection, as a general measure of the effects of drug injection on the health of the animals. It was noted that the mice injected with saline gained weight (average 1.36 g) over the course of the injections, while mice injected with 5-FU or DOXO lost weight (Ave. 0.73 g and 4.24 g, respectively).

For either the Passive Avoidance Test or the Novel Object Recognition Test, there was no significant difference between the mice injected with saline and those injected with 5-FU or Doxorubicin. For example, in the NOR testing, the mean ratio of time spent investigating the novel object over the total time was 0.55 for 5-FU, 0.61 for PBS and 0.59 for Doxorubicin. These results were not found to be statistically significant using a t-test. However, all mice showed signs of anxiety and fearfulness during the testing. It was suggested that the next cohort of mice be handled more prior to the injections.

A second group of mice (group B) was handled every 2-3 days over the course of several weeks prior to the start of injections. In this group, 15 mice each were subjected to 3 injections of saline or cyclophosphamide (50 mg/kg) over the course of one week. Mice were weighed at the beginning and the end of the injection period and were observed daily for signs of sickness or impairment. No signs of impairment or sickness were noted, but mice injected with cyclophosphamide did lose weight (Ave. 1.71 g), while mice injected with saline gained slightly (Ave. 0.25 g). This group of mice was subjected to testing in the Novel Object Recognition and Passive Avoidance Tests. In this cohort, there was a difference in performance on the NOR test, with the drug-treated mice performing somewhat worse than the control mice, but the difference did not reach statistical significance. To try to increase the effects of the drug, we injected mice a fourth time with either saline or cyclophosphamide, while a control group of previously saline-injected animals was not injected again. These animals were again tested using NOR; they had been tested prior to injection to establish a baseline. In this experiment, cyclophosphamide-injected animals performed worse than saline-injected animals (relative to their baseline performance), but unexpectedly, the animals that were not injected again also performed worse. It is difficult to interpret these results.

Because these results have proven to be somewhat disappointing, we will modify the next set of experiments to attempt to produce a larger drug-related effect. We have not yet been able to test the effects of rapamycin treatment on cognitive function (our Aim 2), since we have not yet produced a reproducible cognitive deficit to allow us to measure improvement.

Our current plan is to use different drugs and the previously tested drugs in higher concentration in order to try to produce a larger effect. We are also working with our collaborator to find different behavioral tests that may give more favorable results. If those measures fail, we can measure cell proliferation in the dentate gyrus of the hippocampus following BrdU injections prior to sacrifice and immunohistochemistry. This method should be successful, based on previously published results (Janelins et al, 2010).

Aim 2 of the original proposal is to test the effects of dietary rapamycin treatment on the cognitive functioning of mice following chemotherapy treatments. Since we have not yet established a reliable model system producing reproducible cognitive defects, we have not yet begun this aim.

KEY RESEARCH ACCOMPLISHMENTS

- Testing of dosages of 3 common chemotherapy drugs reported to produce changes in neurogenesis in the dentate gyrus has not yet produced any measurable behavioral changes

REPORTABLE OUTCOMES

No reportable outcomes yet.

CONCLUSION

Thus far, we have not yet produced large enough cognitive changes in mice injected with chemotherapy agents to reproducibly measure using behavioral testing. We will utilize different drugs and/or higher concentrations of the current drugs in the next set of experiments, in order to maximize the chance of seeing an effect. We can also try combinations of drugs to produce larger effects if necessary.

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APPENDICES
None included.

SUPPORTING DATA
None included.