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TITLE: Androgen Deprivation Therapy and Cognitive Impairment

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14. ABSTRACT

Androgen deprivation therapy is used in the treatment of prostate cancer, but an important side effect is impairment of memory and learning. In the hippocampus, a brain region involved in memory and learning, new nerve cells (i.e., neurons) continue to develop throughout adulthood, a process is called adult neurogenesis. The goal of this project is to test the hypothesis that impaired hippocampal neurogenesis underlies the androgen deprivation therapy-induced impairment of cognitive function. We carried out castration surgeries or implanted pellets containing leuprolide (a gonadotropin-releasing hormone analog that reduces plasma testosterone levels) and flutamide (an androgen receptor antagonist). We found that these three approaches, that are used in prostate cancer patients, significantly reduced both the proliferation and survival of new neurons in the hippocampus. These results suggest that androgen-deprivation might cause similar deficits in hippocampal neurogenesis in prostate cancer patients We also found that androgen deprivation does not affect neurogenesis in the subventricular zone, demonstrating that the effects are limited to the hippocampus. Experiments have been completed testing whether the effects can be reversed by drug treatment and the results currently are being analyzed.

15. SUBJECT TERMS

Neurogenesis, neuron, hippocampus, memory, learning, testosterone, androgen, androgen deprivation, castration, prostate cancer, flutamide, leuprolide, proliferation, survival, immunohistochemistry, Western blot.

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INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Androgen deprivation therapy is a well-established treatment for prostate cancer, but an important side effect of androgen deprivation therapy is impairment of memory and learning. In the hippocampus, a brain region that plays a major role in memory and learning, new nerve cells continue to develop throughout adulthood, a process called neurogenesis. The goal of this project is use an animal model to test the hypothesis that impaired hippocampal neurogenesis underlies the androgen deprivation therapy-induced impairment of cognitive function. There are four specific aims. Specific Aim 1 tests the hypothesis that androgen deprivation decreases hippocampal neurogenesis. Specific Aim 2 tests the hypothesis that androgen deprivation disrupts cognitive behavior. Specific Aim 3 tests the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on hippocampal neurogenesis and Specific Aim 4 tests the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on cognitive behavior. The results of the proposed studies could lead to the development of strategies to optimize the physical and mental health of men with prostate cancer and improve the quality of life and well-being of prostate cancer patients and their families.

1. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Neurogenesis, neuron, hippocampus, memory, learning, testosterone, androgen, androgen deprivation, castration, prostate cancer, flutamide, leuprolide, proliferation, survival, immunohistochemistry, Western blot.

2. ACCOMPLISHMENTS:

What were the major goals of the project?

Year 3 Goals (as listed in the approved Statement of Work) and goals carried over from Year 2

Specific Aim 2: To test the hypothesis that androgen deprivation disrupts cognitive behavior.	
Major Task 3: Treat Animals	
Subtask 1: Carry out surgeries and pellet implantation **[4 treatment groups (sham/placebo pellet, castration/placebo pellet, sham/flutamide pellet and sham/leuprolide pellet) x 15 mice/group = 60 mice]**	**13-14**
Milestone(s) Achieved: Surgeries completed	**100% Complete**
Major Task 4: Behavioral Testing, Data Analysis and Manuscript Preparation	
Subtask 2: Behavioral Testing	**16-17**
Milestone(s) Achieved: Behavioral testing completed	**100% Complete**
Subtask 3: Data analysis and manuscript preparation	**17-18**
Milestone(s) Achieved: Data analyzed and manuscript submitted	**90% complete (data analysis complete, manuscript in preparation)**

Specific Aim 3: To test the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on hippocampal neurogenesis.	
Major Task 5: Treat Animals	
Subtask 1: Carry out surgeries and begin drug administration **[6 treatment groups (sham/vehicle, sham/fluoxetine, sham/memantine, castration/vehicle, castration/fluoxetine and castration/memantine) x 2 subgroups (IHC and Western blot studies) x 8 mice/group = 96 mice]**	**19-20**
Milestone(s) Achieved: Surgeries and drug administration completed	**100% complete**
Major Task 6: Sacrifice Animals, Tissue Processing and Data Analysis	
Subtask 1: Sacrifice Animals	**22**
Milestone(s) Achieved: Animals sacrificed	**100% complete**
Subtask 2: Process tissue	**22-27**
Milestone(s) Achieved: Tissue processing completed	**70% complete**
Subtask 3: Data analysis	**27-28**
Milestone(s) Achieved: Data analysis completed	**50% complete**

Specific Aim 4: To test the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on cognitive behavior.	**Experiments not initiated**
Major Task 7: Treat Animals	
Subtask 1: Carry out surgeries and begin drug administration **[6 treatment groups (sham/vehicle, sham/fluoxetine, sham/memantine, castration/vehicle, castration/fluoxetine and castration/memantine) x 15 mice/group = 90 mice]**	
Milestone(s) Achieved: Surgeries and drug administration completed	
Major Task 8: Behavioral Testing, Data Analysis and Manuscript Preparation	
Subtask 2: Behavioral Testing	
Milestone(s) Achieved: Behavioral testing completed	
Subtask 3: Data analysis and manuscript preparation	
Milestone(s) Achieved: Data analyzed and manuscript submitted	

What was accomplished under these goals?

During this reporting period we completed the tasks left over from the second reporting period. Specifically, we completed the surgeries and pellet implantation (Major Task 3/Subtask 1), the behavioral testing (Major Task 4, Subtask 2) and the analysis of the behavioral data (Major Task 4, Subtask 3); and we are in the progress of preparing a manuscript for publication. Further, we completed the surgeries and drugs administration (Major Task 5, Subtask 1) and sacrificed the animals (Major Task 6, Subtask 1). Currently, we are finishing up processing the tissue and analyzing the data from this task.

Based upon immunohistochemistry and Western blot analyses, we found that that all three approaches to producing androgen deprivation (castration, the gonadotropin-releasing hormone analog leuprolide and the androgen receptor antagonist flutamide) significantly reduced both the proliferation and survival of new neurons in the subgranular zone of the dentate gyrus of the hippocampus. We have now determined that neurogenesis in the subventricular zone, another region where adult neurogenesis occurs, is not affected by androgen deprivation. We are looking forward to the outcome of the analyses of the data obtained in Specific Aim 3 which will demonstrate whether the androgen deprivation-induced disruption of hippocampal neurogenesis can be blocked by treatment with fluoxetine and/or memantine.

Rigorous analysis of the behavioral data obtained in Specific Aim 2 showed that androgen deprivation did not affect behavior. This conclusion was based on negative data obtained by assessing performance on spontaneous alternation, a test that indicates deficits in spatial working memory, and the Barnes maze, a test that assesses hippocampal-dependent spatial memory. We had modified the protocol (with IACUC and ACURO approval) and added a third test, the Novel Object Recognition Task, that also evaluates spatial memory. We also obtained negative results using this test. Disruption of adult hippocampal neurogenesis is thought to be linked to deficits in spatial memory, and patients with cognitive impairment after androgen-deprivation therapy have problems with memory. Our results were not expected and there could be several explanations. First, the effects on behavior could be too subtle to detect using these tests of spatial memory. Other more sensitive tests could be used. It is important to point out that the effects in human are rather subtle in nature. Second, patients who have prostate cancer and undergo androgen deprivation therapy tend to be older, whereas our experimental animals were young. Therefore, the animal model might not have been appropriate. Normal age-related deficits in prostate cancer patients could have additive or synergistic effects with the effects of androgen deprivation on memory. Studies could be carried out in old mice to test whether this could be true. Third, it is possible that androgen deprivation-induced decreases in neurogenesis might not occur in and/or such decreases might be linked to memory deficits in humans. Because of the lack of effect on behavior, we chose not to pursue the behavioral experiments in Specific Aim 4 because it would not be a productive use of time and effort.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

The results were/will be disseminated by the following poster presentations:

1. "The Effects of Androgen Deprivation Therapy on the Adult Hippocampal Neurogenesis and Cognition in Mice." Alkam, T., Atkinson, K., Jo, J., Chan, J., Smith, E. and Pechnick, R.N. Presented at the annual meeting of the Society for Neuroscience, San Diego, CA, November, 2018.

2. "Androgen Deprivation Disrupts Adult Hippocampal Neurogenesis". Alkam, T., Jo, J. and Pechnick, R.N. Presented at the 7th Mediterranean Neuroscience Conference, Marrakech, Morocco. June 2019.

3. "Disruption of Adult Hippocampal Neurogenesis Following Androgen Deprivation". Alkam, T., Jo, J., Shota, R., Oh, D., Alquisola, A. and Pechnick, R.N. Abstract submitted, to be presented at the annual meeting of the American College of Neuropsychopharmacology, Orlando, FL, December, 2019.

What do you plan to do during the next reporting period to accomplish the goals?

Currently, we are on a no-cost time extension through 7/31/2020. We are now focused on tissue processing, data analysis and manuscript preparation. During the next period we will complete Major Task 4/Subtask 3, Major Task 5/Subtask 2 and Major Task 5/Subtask 3. All manuscripts will be submitted for publication. We expect to be able to complete all of the goals and objectives by the end of the no-cost extension.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our finding that all three treatments reduce both neuronal proliferation and survival in the subgranular zone of the dentate gyrus of the hippocampus are significant because all three methods are used in the treatment of prostate cancer in humans. This suggests that patients might show similar deficits in hippocampal neurogenesis. This supports our underlying hypothesis that hypothesis that impaired hippocampal neurogenesis underlies the androgen deprivation therapy-induced impairment of cognitive function in patients suffering from prostate cancer. If we find that drugs that stimulate neurogenesis reduce or block the effects of androgen deprivation (to be determined in Specific Aims 3), this could lead to the development of new treatments androgen deprivation-induced cognitive impairment in prostate cancer patients.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

None

Actual or anticipated problems or delays and actions or plans to resolve them

Although we tried different interventions, we continued to have problems with mice fighting, having to be deleted from the experiment and requiring ordering replacement animals. This delayed our progress. After further research, consultation with our veterinarian and the supplier, we began to order litter mates for each experiment. This solved the problem and we did not encounter any more fighting. However, litter mates are more costly and they also take longer for delivery, i.e., 5-6 weeks. At this point all animal work has been completed and we are focused on tissue processing, data analysis and manuscript preparation.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

1. "The Effects of Androgen Deprivation Therapy on the Adult Hippocampal Neurogenesis and Cognition in Mice." Alkam, T., Atkinson, K., Jo, J., Chan, J., Smith, E. and Pechnick, R.N. Presented at the annual meeting of the Society for Neuroscience, San Diego, CA, November, 2018.

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Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Robert N. Pechnick, Ph.D. – no change

Tuerxun Ailikemu, M.D., Ph.D. – no change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

N/A

QUAD CHARTS:

N/A

9. APPENDICES:

N/A