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TITLE: Androgen Deprivation Therapy and Cognitive Impairment

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

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 (i.e., neurons) continue to develop throughout adulthood, a process is called 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 surgeries (castration and sham castration) and implanted placebo pellets and pellets containing leuprolide (a gonadotropin-releasing hormone analog that reduces plasma testosterone levels) and flutamide (an androgen receptor antagonist). We found that all three approaches to producing androgen deprivation significantly reduced both the proliferation and survival of new neurons in the hippocampus. These results are significant because all three methods are used in the treatment of prostate cancer in humans, and suggest that patients might show similar deficits in hippocampal neurogenesis. During the second year we initiated behavioral testing. The testing is continuing and the data 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 2 Goals As Listed In Approved Statement of Work (SOW)

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	**14 (80% Complete)**
Major Task 4: Behavioral Testing, Data Analysis and Manuscript Preparation	
Subtask 2: Behavioral Testing	**16-17**
Milestone(s) Achieved: Behavioral testing completed	**17 (80% Complete)**
Subtask 3: Data analysis and manuscript preparation	**17-18**
Milestone(s) Achieved: Data analyzed and manuscript submitted	**18 (80% Complete)**

Specific Aim 3: To test the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on hippocampal neurogenesis.	Not yet started
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	**20**
Major Task 6: Sacrifice Animals, Tissue Processing and Data Analysis	
Subtask 1: Sacrifice Animals	**22**
Milestone(s) Achieved: Animals sacrificed	**22**

What was accomplished under these goals?

During this reporting period we completed tasks left over from the first reporting period. Specifically, we completed the processing of the tissue (Major Task 2/Subtask 2) and data analysis (Major Task 2/Subtask 3). 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. In the immunohistochemistry studies, Ki-67 labels proliferating cells. Compared to placebo-treated subjects, all three treatments reduced Ki-67 labeling, demonstrating reduced neuronal proliferation in the hippocampus (Table 1). NeuN/BrdU double labeling indicates the number of cells that survive post-proliferation. Compared to placebo-treated subjects, all three treatments reduced NeuN/BrdU double labeling, demonstrating reduced neuronal survival. Therefore, all three treatments reduce both neuronal proliferation and survival in the subgranular zone of the dentate gyrus of the hippocampus. This is a new finding.

Table 1. Immunohistochemistry Results Summary

	Sham + placebo	Castration+ placebo	Sham + Leuprolide	Sham + Flutamide
Ki-67	1990 ± 134	1389 ± 82.5 **	1212 ± 118.2 ***	1353 ± 68.31***
$NeuN^+/BrdU^+$	81 ±10.73	37.83 ± 10.55 **	30 ± 4.47 **	30 ± 6.83 **

Ki-67: the number of cells that positive for Ki-67 in dentate gyrus of the hippocampus; **p<0.01 *vs*. sham + placebo group; ***p<0.001 *vs*. sham + placebo group; $NeuN^+/BrdU^+$: the number of cells that positive both for $NeuN^+$ and $BrdU^+$ in the dentate gyrus of the hippocampus; **p<0.01 *vs*. sham + placebo group. Mean ± SE, N=8.

The results from the Western Blot studies of the whole hippocampi confirmed the immunohistochemistry findings (Table 2). All three treatments reduced levels of Ki-67 protein expression, verifying reduced proliferation. DCX is expressed in immature neurons, and NeuN is expressed in mature neurons. We did not find differences in levels of DCX and NeuN expression as a consequence of androgen deprivation. We believe that this is because the Western Blot analyses use the entire us whereas the immunohistochemistry studies focus only on the subgranular zone of the dentate gyrus of the hippocampus. The changes in the dentate gyrus might be diluted out when the whole hippocampus is evaluated.

Table 2. Western Blot Results Summary

	Sham + placebo	Castration+ placebo	Sham + Leuprolide	Sham + Flutamide	Significance
Ki-67	1 ± 0.03	0.83 ± 0.04**	0.76 ± 0.06***	0.85 ± 0.04**	**P<0.01; ***P<0.001
DCX	1 ± 0.08	0.92 ± 0.05	0.96 ± 0.07	0.98 ± 0.05	p>0.05
NeuN	1 ± 0.10	0.97 ± 0.06	1 ± 0.06	0.99 ± 0.10	p>0.05

Levels of protein expression (normalized by GAPDH expression in each sample) in “sham + placebo” group are regarded as the standard. Levels of protein expression in other treatment groups are divided by that of “sham + placebo” group to find "fold-changes". Mean ± SE, N=11.

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. Furthermore, the results support and set the groundwork for the experiments described in Specific Aims 3 and 4.

We also began Specific Aim 2, which tests the hypothesis that androgen deprivation disrupts cognitive behavior, and testing is near completion. We decided to add an additional behavioral test, the Novel Object Recognition Task. This task evaluates spatial memory and will provide supportive information on the behavioral effects of androgen deprivation. It will be used in conjunction with the Y- and Barnes maze procedures that were approved previously. The addition of the Novel Object Recognition Task required a modification of our Institutional IACUC. After this modification was approved by our IACUC, we applied for approval of the modification through ACURO, and approval was granted on 5/6/2018.

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 will be disseminated via the following poster presentation that will be given at the annual meeting of the Society for Neuroscience, San Diego, CA, November, 2018: "The Effects of Androgen Deprivation Therapy on the Adult Hippocampal Neurogenesis and Cognition in Mice." Tursun Alkam, Kelley Atkinson, Jonathan Jo, Joshua Chan, Ekaterina Smith, Robert N. Pechnick.

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

During the next reporting period we will complete the treatment of the animals (Major Task 3), the behavioral testing behavioral tests of the animals (Major Task 4/Subtask 2) and data analysis (Major Task 4/Subtask 4). We will begin Specific Aim 3, which tests the hypothesis that that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on hippocampal neurogenesis, and Specific Aim 4, which tests the hypothesis that drugs that increase hippocampal neurogenesis will reduce the effects of androgen deprivation on cognitive behavior. We will continue to follow the approved SOW and expect to be able to complete all of the goals and objectives by the end of the next reporting period.

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 and 4), 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

As mentioned above, we added an additional behavioral test, the Novel Object Recognition Task. This task evaluates spatial memory and will provide supportive information on the behavioral effects of androgen deprivation. It will be used in conjunction with the Y- and Barnes maze procedures that were approved previously. The addition of the Novel Object Recognition Task required a modification of our Institutional IACUC. After this modification was approved by our IACUC, we applied for approval of the modification through ACURO, and approval was granted on 5/6/2018. The Novel Object Recognition Task is carried out

after the subjects complete Barnes maze testing. Therefore, no new subjects are required and not additional costs are incurred. The additional of this new test lengthens that behavioral testing period by less than one week.

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

We encountered two problems. First, initially we found that some mice in the leuprolide-treated group had a tendency to fight. In some cases the wounds were severe enough that the subjects were deleted from the experiment and required ordering replacement animals. In consultation with our resident Veterinarian, we concluded that this might be due to a surge in testosterone and associated aggressiveness that can occur after the initiation of treatment with leuprolide. We now monitor the mice on a more regular basis and we have not had further problems. Second, initially we had planned on using a sample size of 8 for the Western blot experiments. Due to variability among subjects, we found that he had to increase the sample size to 11 to obtain statistically significant differences among treatment groups.

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

The following poster presentation: "The Effects of Androgen Deprivation Therapy on the Adult Hippocampal Neurogenesis and Cognition in Mice." Tursun Alkam, Kelley Atkinson, Jonathan Jo, Joshua Chan, Ekaterina Smith, Robert N. Pechnick. To be presented at the annual meeting of the Society for Neuroscience, San Diego, CA, November, 2018.

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