Drug Combinations Modulate Stem Cell Proliferation in the Subventricular Zone of 11-Month-Old Rats: Innate Gender Differences Could Explain Alzheimer's Susceptibility.

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Abstract

Various drugs and drug combinations were tested for their ability to modulate the stem cell proliferation in the Subventricular zone (SVZ) of 11 month Sprague Dawley rats. Female rats showed low endogenous stem cell proliferation, significantly different from male rats (P<0.0001). The combination of Simvastatin, Ascorbic Acid and Fluoxetine significantly increased stem cell proliferation in the SVZ in female rats. In the male rats, Fluoxetine significantly decreased stem cell proliferation in all regions of the SVZ (anterior, middle, and posterior). When comparing the stem cell proliferation in different SVZ regions across the two genders with a 2-way ANOVA, we found that fluoxetine and simvastatin significantly decreased male stem cell proliferation compared to females, but the drug combinations of fluoxetine and statin removed these sex differences. The drug combination of Simvastatin, Ascorbic Acid and Fluoxetine (SAAF) increased female stem cell proliferation over male, and returned male stem cell proliferation back to endogenous levels: this drug combination might be able to delay onset of a neurodegenerative disease, such as Alzheimer's disease.

Methods

Male and Female Sprague Dawley rats received different drugs or a vehicle daily for a total of thirty days. All animals received their drugs around noon, encased in a 4 gram ball of sugar cookie dough, and they generally voluntarily ate the drugs within about 5 minutes (Corbett et al, 2012).

At the end of the study, the rats were cardioperfused with phosphate buffered saline (PBS), followed by 4% paraformaldehyde in phosphate buffered saline. The brain was dissected out and post-fixed in 4% paraformaldehyde for 24 hours. The brain was then placed in 30% sucrose for three days, in preparation for cryosectioning. Fifty micron coronal sections were cut with a cryostat and collected into PBS. Free floating coronal brain sections were placed in blocking solution (PBS with 0.3% Tween and 3% goat serum) for one hour. We incubated overnight at 5 degrees C with a 1:1000 dilution of AbCam anti-Ki67 (AB15580) antibody. We washed twice with PBS-Tween, then incubated for one hour with biotinylated secondary antibody (goat-anti-rabbit IgG) from a Vector ABC kit. We then washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (avidin, horse-radish peroxidase). The sections were washed twice with PBS-Tween and the substrate DAB was used to visualize antibody staining (10 minute incubation) and the sections washed and mounted onto gel-coated microscope slides. Sections were coverslipped with DPX mountant.

Pictures of the Ki67 staining in the subventricular zone of the lateral brain ventricles were digitally taken on a brightfield microscope. Images were montaged (if necessary), to get all of the ventricular staining into a single picture. The staining area was measured using Image J.

Graphs of the stem cell proliferation were made using GraphPad Prism Software. Statistical methods used included 1 way ANOVA (Figures 4 and 5) and 2 way ANOVA (Figures 3), analyzed with the Prism software.

Figure 1.

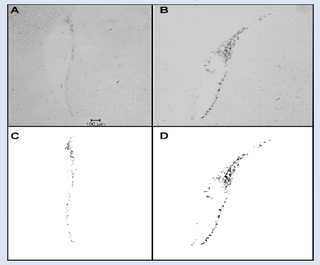

Figure 1. Representative images of Ki67 staining in anterior ventricles of 11 month old female rats. Panel A shows the normal Ki67 staining (no drugs) and the respective mask used to measure the area of Ki67 staining in Image J from panel A is shown in panel C. Panel B showing Ki67 staining in the anterior ventricle of an 11 month old female rat in response to a 30 day drug treatment to SAAF (simvastatin, ascorbic acid and fluoxetine), with the respective mask used to determine Ki67 staining area shown in panel D. Scale bar in panel A shows 100 micrometers.

Figure 2.

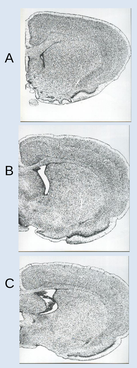

Figure 2.For each ventricle we first determined location of the ventricle (anterior, middle or posterior), then indicated the average Ki67 staining area per slice for all of the replicate slices within that region for one particular rat.

In figure 5, for each of female rats we had an average of 7.5 sections analyzed for the anterior Subventricular zone, an average of 12 sections analyzed for the middle Subventricular zone, and an average of 5.8 sections analyzed for the posterior Subventricular zone.

In Figure 4, for each of the male rats we had an average of 8.8 sections analyzed for the anterior Subventricular zone, and average of 16 sections analyzed for the middle Subventricular zone and an average of 5 sections analyzed for the posterior Subventricular zone.

Figure 3. Male vs Female Stem Cell Proliferation by Single Drugs in the SubVentricular Zone

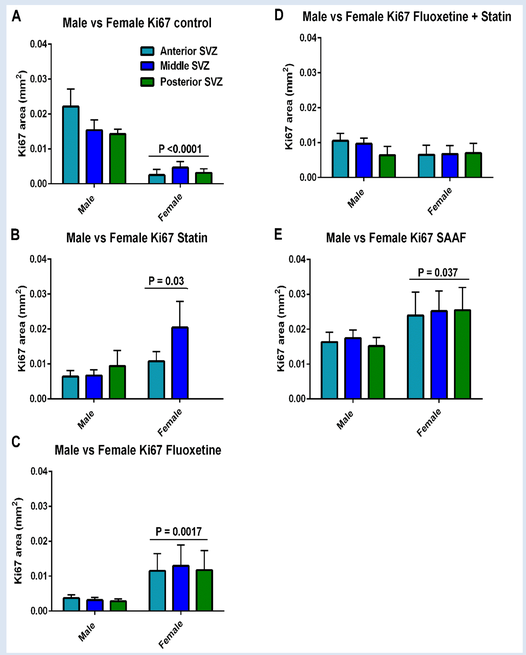

Figure 3. Each bar represents the Mean Ki67 staining area and Standard Error of the Mean for male versus female rats, separating the Subventricular zone into anterior (light gray), middle (gray) and posterior (black) regions. Significant differences are shown by a line over the significantly different group with the P value on top of the line (2-way ANOVA). Panel A represents 11 month old control rats (no drugs). Panel B represents rats that were given 30 days of 1 mg/kg simvastatin. Panel C represents rats that were given 30 days of 5 mg/kg fluoxetine. Panel D represents rats that were given a combination of 5 mg/kg fluoxetine and 1 mg/kg simvastatin for 30 days. Panel E represents rats that were given a combination of SAAF (1 mg/kg simvastatin, 20 mg/kg Ascorbic Acid, and 5 mg/kg Fluoxetine) for 30 days.

Figure 4: Drug Combos effect on Male Stem Cell Proliferation in the SVZ

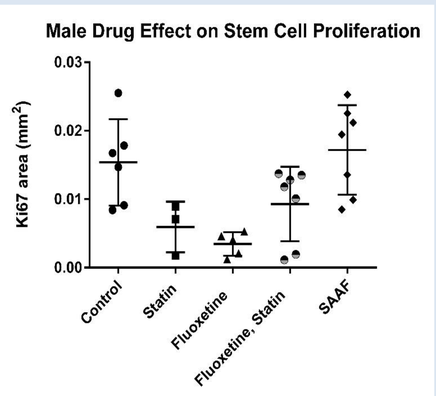

Figure 4. Each symbol represents the mean area of Ki67 staining in the Subventricular zone taken from 30 coronal brain sections (50 micrometer thickness) for a single rat. The mean for the group is indicated by the wide horizontal bar and the error bar represents the standard deviation for the group. Fluoxetine significantly decreased stem cell proliferation compared to both control and SAAF (one way ANOVA)

Figure 5. Drug Combos Effect on Female Stem Cell Proliferation in SVZ

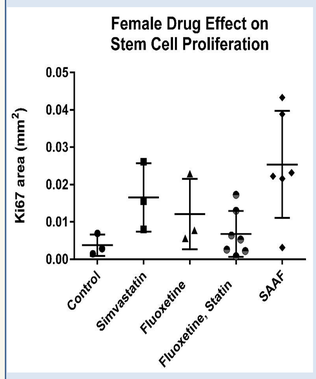

Figure 5: Each symbol represents the mean area of Ki67 staining from the Subventricular zone from 25 coronal brain sections taken for a single rat. The mean for the group is indicated by the wide horizontal bar and the error bar represents the standard deviation for the group. Statistical test was one way ANOVA with SAAF treatment increasing stem cell proliferation statistically over control (P=0.020).

Conclusions

Post-menopausal female rats have significantly lower stem/progenitor cell proliferation in the Subventricular Zone of the Lateral Ventricles compared to males, which may explain why females are more susceptible to a neurodegenerative disease such as Alzheimer's disease. We present evidence that stem/progenitor cell proliferation may be rescued, in both 11 month old males and females, by the drug combination of simvastatin, ascorbic acid and fluoxetine suggesting that this drug combination could have a therapeutic potential in treating Alzheimer's disease.

References

1. **Corbett, A**., McGowin, A., Sieber, S., Flannery, T., and Sibbitt, B. 2012. A method for reliable voluntary oral administration of a fixed dosage (mg/kg) of chronic daily medication to rats. **Laboratory Animals** 46 (4): 318-324
2. Swanson, L.W. (1992). *Brain Maps: Structure of the Rat Brain.* Amsterdam, The Netherland: Elsevier Science Publishers B.V.