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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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 volitionally 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 then incubated with a 4% Triton X-100 solution and 3% goat serum for one hour, then incubated with primary antibody. We washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (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 (goat anti-rabbit IgG). A Vector ABC kit. We then washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (goat anti-rabbit IgG). A Vector ABC kit. We then washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (goat anti-rabbit IgG). A Vector ABC kit. We then washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (goat anti-rabbit IgG). A Vector ABC kit. We then washed twice with PBS-Tween, then incubated section for 45 minutes with the ABC reagent (goat anti-rabbit IgG). A Vector ABC kit. We then washed twice with PBS-Tween, then incuba

Figures

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. In Figure 2 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 each rat. In Figure 5, for each of female rats we had an average of 1.3 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.

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 an error bar. 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 4. Drug Combinations Effect on Female Stem Cell Proliferation in SVZ

Figure 5. Drug Combinations Effect on Male Stem Cell Proliferation in SVZ

Conclusions

Post-menopausal female rats have significantly lower stem/progenitor cell proliferation in the Subventricular Zone of the Lateral Ventrices 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