Timing of Fluoxetine Administration Post-Stroke Impacts Infarct Volume and Hemorrhagic Transformation

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Introduction

- Previous experiments have indicated that administration of Fluoxetine and Simvastatin at 20-26 hours post-stroke decreases the volume of ischemic infarcts in Sprague Dawley female rats (10-12 months old)[1]. This experiment more closely modeled clinical trials by starting all animals on Simvastatin 7 days pre-stroke and continuing daily. Daily Fluoxetine and ascorbic acid administration were begun at three initiation time points (6-12 hours post-stroke, 20-26 hours post-stroke and 48-54 hours post-stroke).

- Adult male rats (10-12 months) who were treated 20-26 hours post-stroke with Fluoxetine and ascorbic acid showed both significant decrease in infarct volume (7.389±1.661 mm^3 SEM, P=0.0098) and a reduced relative risk of hemorrhagic transformation

Methods

- All male retired breeder Sprague-Dawley rats were on 1mg/kg Simvastatin for 7 days prior to stroke induction and continued for 7 days post-stroke, then euthanized.
- Ischemic strokes were induced by endothelin-1 injection, targeting the forelimb motor cortex.
- Control group: no Fluoxetine or ascorbic acid post stroke treatment.
- Daily Fluoxetine (5 mg/kg) and ascorbic acid (20 mg/kg) treatment was begun at three different time points post stroke: 6-12 hrs., 20-26 hrs. or 48-54 hrs. following stroke induction.
- Drug was delivered using a voluntarily oral administration developed method[2].
- All animals were euthanized on post-stroke day 7, so we could see any evidence of hemorrhagic transformation.
- Coronal sections of brain stained with hematoxylin and eosin (H&E) and infarct volumes and hemorrhagic transformation were assessed.

Results

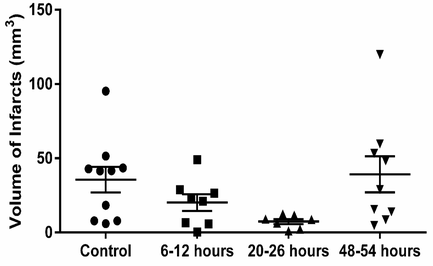

Figure 1. Infarct volume varies with timing of Fluoxetine, and ascorbic acid administration after ischemic stroke induction. All animals were started on 1mg/kg Simvastatin 7 days pre-stroke and continued throughout the study. Direct comparison of infarct volumes between all groups treated with Simvastatin only or Fluoxetine, ascorbic acid treatment at different time-points after stroke induction. When outliers removed, infarct volumes showed a statistically significant reduction in the 20-26 hours treatment group (P=0.0098; One-way ANOVA).

	Hemorrhagic Transformation	No Hemorrhagic Transformation	Relative Risk (RR)
6-12 hours	5	3	1.0417 (95% CI=0.4981 to 2.1783)
20-26 hours	2	7	0.3704 (95% CI=0.0987 to 1.3905)
48-54 hours	5	4	0.9259 (95% CI=0.4274 to 2.0059)
Control	6	4	

Table 1. The relative risk (RR) of developing hemorrhagic transformation in treatment groups given Fluoxetine and ascorbic acid at different time-points. Note: the two hemorrhagic transformations in the 20-26 hour group were statistical outliers in the infarct volume graph above.

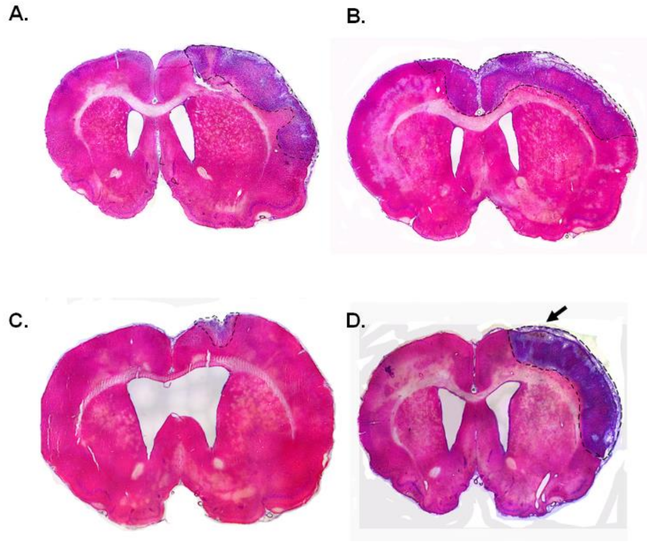

Figure 2. Representative images showing H&E stained coronal brain sections with infarcts. A. Control (Simvastatin only) administered 7 days pre-stroke and 7 days post-stroke shows large infarct volume with hemorrhagic transformation. **B.** Infarct volume was unchanged if Fluoxetine and ascorbic acid was administered 6-12 hours after stroke and had highest relative risk of hemorrhagic transformation **C.** Significant reduction of both infarct volume and hemorrhagic transformation when Fluoxetine, ascorbic acid combination was administered 20-26 hours after stroke surgery **D.** Panel shows same infarct size when drug combination was delayed 48-54 hours; a possible bleeding (hemorrhagic transformation) is marked with an arrowhead.

Conclusions

- This study was designed to match the protocol in previous and ongoing clinical trials testing Fluoxetine effects on motor recovery, while adding two earlier drug administration time-points.
- In male rats already on Simvastatin, giving Fluoxetine beginning 48 hours after stroke had no impact on infarct volume and hemorrhagic transformation, similar to the FLAME Clinical Trial[3]
- If Fluoxetine was given 6-12 hours after stroke induction, there is substantial hemorrhagic transformation and the infarct volume is not significantly decreased.
- If Fluoxetine was given 20-26 hours post-stroke, then the infarct volume was significantly decreased and the relative risk of hemorrhagic transformation substantially reduced.
- The results are similar to the results of the previous experiment in our lab, in which female Sprague-Dawley rats was used.

References

1. Balch M.H, Ragas M.A, Wright D, Hensley A, Reynolds K, Kerr B and Corbett AM, *Appropriate Timing of Fluoxetine and Statin Delivery Reduces the Risk of Secondary Bleeding in Ischemic Stroke Rats*. Journal of Neurology and Neuroscience, 2015
2. Corbett A, McGowin A, Sieber S, Flannery T, Sibbitt B. *A method for reliable voluntary oral administration of a fixed dosage (mg/kg) of chronic daily medication to rats*. Lab Anim. 2012;46:318-32
3. Chollet et al, Fluoxetine for Motor Recovery after Acute Ischemic Stroke (FLAME): a Randomized Placebo Controlled Trial. 2011 Lancet Neurol 10: 123-130