Stroke Physical Rehabilitation: Impact on Motor Functional Recovery in Drug-Treated versus Vehicle-Treated Rats

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Methods

Endothelin-1-induced Ischemic Stroke

The procedure of endothelin-1-induced cerebral ischemic stroke is based on modifications to the procedure from Winkle et al. (2008). Procedure begins by inducing anesthesia using IP injections of 100 mg/kg ketamine and 10 mg/kg xylazine. The rat is then placed in a stereotaxic apparatus using non-traumatic ear bars. A micro-drill was used to drill two holes in the skull where 1.5 μl (80% propylene) endothelin-1 (human and porcine, EM Chemicals) is injected at a concentration of 400 pmol/l. Infarct volume is determined using high resolution micro-CT imaging at day 3.

Voluntary Oral Drug Administration

The drug vehicle was 4 grams of purchased Pilsbury® sugar cookie dough. The cookie dough was weighed, mixed into a ball and then a depression pushed into the ball with a finger. The individually weighed drug (Pharmacological grade crushed tablets or contents of capsules) were placed into the depression in the dough ball and rolled with the fingers to ensure the drug is evenly distributed throughout. The dough ball was placed into a clear plastic box and the rats were given 20 minutes to eat the cookie. This procedure was completed daily for a total of 91 days. Animals were euthanized on post-stroke day 92. Because this early drug delivery (6-12 hours) caused a previously unseen increase in infarct size in animals treated with Fluoxetine and in individually housed rats, this study was compared with animals from a previous study which had received the drugs 24-48 hours after stroke induction and continued daily for a total of 30 days.

Table 1. Treatment Groups and Rehabilitation. All animals in this study, the drugs were administered 6-12 hours after cerebral stroke induction and continued daily for a total of 91 days. All animals were euthanized on post-stroke day 92. Because this early drug delivery (6-12 hours) caused a previously unseen increase in infarct size in animals treated with Fluoxetine and in individually housed rats, this study was compared with animals from a previous study which had received the drugs 24-48 hours after stroke induction and continued daily for a total of 30 days.

Rat

Group Treatment Rehabilitation Duration of Rehabilitation

1. 14

Vehicle Control

No

31 days

2. 14

1 mg/kg Simvastatin

Yes

31 days

3. 14

5 mg/kg Fluoxetine + 1 mg/kg Simvastatin

Yes

31 days

4. 14

1 mg/kg Simvastatin + 1 mg/kg Fluoxetine

Yes

31 days

Table 2. Treatment Groups and Rehabilitation. All animals in this study, the drugs were administered 6-12 hours after cerebral stroke induction and continued daily for a total of 91 days. All animals were euthanized on post-stroke day 92. Because this early drug delivery (6-12 hours) caused a previously unseen increase in infarct size in animals treated with Fluoxetine and in individually housed rats, this study was compared with animals from a previous study which had received the drugs 24-48 hours after stroke induction and continued daily for a total of 31 days.

Rehabilitation:

Rehabilitation was begun on post-stroke day 8 and continued every other day until post-stroke day 45. During the rehabilitation, the hanging shelve was changed every 3 days and continued through the end of the rehabilitation period. Previous work showed our drug combination greatly enhanced neurogenesis, which is impaired in rodents by stress, and physical restraint of the unimpaired limb would be a major stressor for the rats. This hanging shelve rehabilitation accomplished rehabilitation of the impaired limb without restraint, thus reducing stress during the rehabilitation.

Conclusions

1. The drug combination (Fluoxetine and Simvastatin) treatment achieved slightly greater motor functional recovery in rehabilitated animals compared to what was observed in the non-rehabilitated animals.

2. Rehabilitation did not improve the functional recovery seen in the drug-treated animals.

3. Infarct volumes were significantly larger when the drug combination is given at an early time point (6-12 hours after stroke induction) versus a later time point (24-48 hours after stroke induction). Earlier treatment may lead to a secondary hemorrhagic stroke.

References


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Abstract

Post-stroke physical rehabilitation has been considered essential for enhancing motor functional recovery. In this study, adult female Sprague-Dawley rats were subjected to cerebral ischemic stroke induced by endothelin-1, then treated using either a unique drug combination of 5 mg/kg Fluoxetine and 1 mg/kg Simvastatin or a vehicle control beginning 6-12 hours after stroke induction. On post-stroke day 8, the rats were subjected to voluntary physical rehabilitation every other day for a period of five and half weeks. The aim of this study is to determine whether physical rehabilitation might enhance motor functional recovery in drug-treated versus vehicle-treated rats. In drug-treated rats, both rehabilitated and non-rehabilitated groups showed a 34% recovery in contralateral limb function; whereas, in vehicle-treated rats, animals who underwent physical rehabilitation showed a mean of 26% recovery in contralateral limb function versus 18.6% recovery in non-rehabilitated ones, which were both significantly different from drug-treated groups. On the other hand, the palmar lateral of non-rehabilitated (control and drug-treated) rats showed a significant (-23% versus -0.1%) difference in recovery from rehabilitation. The difference in recovery from rehabilitation, was also a significant difference in the baseline (palmar lateral) deficit between rehabilitation and non-rehabilitation groups which might explain this statistical difference in the palmar lateral limb recovery. The starting time for drug delivery may have significantly influenced the functional recovery. The early delivery (6-12 hours after stroke induction) of this drug combination resulted in a larger intact size than had previously seen when this drug treatment was delivered 24-48 hours after stroke induction, possibly due to a secondary hemorrhagic stroke.

This study proves that our post-stroke drug combination treatment allows for motor functional recovery in those individuals for whom physical rehabilitation may not be possible, giving slightly better motor recovery in grasping.

Figure 1: Voluntary Physical Rehabilitation

A. Hanger designed for rehabilitation. This hanger shelve to hold peanut butter just outside of an operant bar. B. The rat is only able to reach to the contralateral paw to get peanut butter. C. The rat is hanged every other day beginning 8 days post-stroke and continuing for an overall period of 5.5 weeks. If animals refused to eat from the shelve, they were put into the No Rehabilitation group. All other animals generally began to eat from the shelves within three weeks. We could clearly determine if the animal underwent rehabilitation treatment has been taken from the shelve, whereas, in C. the animal has partially eaten from the shelve. In D. The animal has completely cleaned the shelve of all peanut butter. We saw no difference in the number of completely versus partially-cleaned shelves between the drug groups. This method enabled physical rehabilitation of the contralateral limb without the stress of physical restraint of the ipsilateral limb.

Figure 2: Montoya Staircase (Assessment of Functional Recovery)

A. The rats are allowed only to reach the platform with the arm that has been previously treated. B. Any difference found between the No rehabilitation group and the Non-rehabilitation group. C. Rehabilitation Effect on Contralateral Functional Recovery. The percentage of contralateral functional recovery (28%) in the Contralateral rehabilitation group (hatched yellow bar) is enhanced compared to the Non-rehabilitation group (8%, solid yellow bar). However, there was no significant difference in the percentage of contralateral functional recovery between the Fluoxetine A Simvastatin (FS) Rehabilitation group (hatched green bar) and the FS Non-rehabilitation group (solid green bar), where both are showing 34% cerebral recovery. There is a significant difference between drug-treated groups and control group (P = 0.007) and a significant difference between Non-rehabilitation groups with drug treatment versus control group (P = 0.041). C. Rehabilitation Effect on Infarct Volume. The percentage of ipsilateral functional recovery in both Control and Drug Non-rehabilitation groups (solid yellow and green bars; -23%) compared to the Control and Drug Rehabilitation groups (hatched yellow and green, -0.1%) is statistically significant (P = 0.05). E. Montoya Staircase Post-stroke Baseline Functional Deficit. The graph shows statistically significant functional deficit in post-stroke contralateral sides of both Rehabilitation and Non-Rehabilitation animals (approx. 55% and 66% functional deficit respectively (P = 0.001) compared to the ipsilateral side, but no significant difference between Rehabilitation and Non-Rehabilitation baseline deficits for contralateral functional. The ipsilateral side baseline deficit (63%) of the Non-Rehabilitation group was found to be significantly different from the Rehabilitation (1%) (P = 0.001). This may have been caused by slight differences in the depth of endotracheal injection in the stroke induction surgeries, causing the rat to extend into the corpus callosum.

Figure 3: Infarct Size and Recovery in Animals with No Rehabilitation

A. Infarct volumes at post-stroke day 92 (solid bars) and post-stroke day 32 (hatched bars) are compared for animals that had same stroke induction procedure, but vary in the starting point of FS vehicle delivery. We are comparing two different time points for the same treatment; however; in general, infarct volumes are known to decrease in size with time, as edema is resolved. In the solid colored bars, the drug or vehicle was delivered 6-12 hours after stroke induction. In the hatched colored bars, the drug or vehicle was delivered 20-26 hours after stroke induction. There is a significant difference (P = 0.016) in the effect of the Fluoxetine and Simvastatin on the infarct volume depending on when the drug is first administered. A later delivery time is more beneficial when considering the final infarct volume. There is no significant difference in the control and non-treatment groups based on the drug delivery time.

The optimal infarct volume for greater than 20% recovery with the drugs appears to be between 8 and 22 mm3 in the group of animals. The very large infarct volumes associated with the drug treatment (greater than 30 mm3) suggest that early drug delivery may result in secondary hemorrhagic stroke.