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

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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 8.6% recovery in non-rehabilitated ones, which were both significantly different from drug-treated groups. On the other hand, the ipsilateral limb of non-rehabilitated (control and drug-treated) rats showed a significant (~23% versus -0.1%) difference in recovery from rehabilitated rats; there was also a significance difference in the baseline ipsilateral deficit between rehabilitation and non-rehabilitation groups which may help explain this statistical difference in the ipsilateral limb recovery. The starting time for drug delivery may have negatively influenced the functional recovery. The early delivery (6-12 hours after stroke induction) of this drug combination resulted in a larger infarct size than had previously been seen when this drug treatment was delivered beginning 20-26 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.

Methods

Endothelin-1-induced Ischemic Stroke:

The procedure of endothelin-1 induced cerebral ischemic stroke is based on modifications to the procedure from Windle *et al.* (2006)[1]. Procedure begins by inducing anesthesia using 5% isoflurane . Anesthesia was maintained with 2-2.5% isoflurane by using an inhalation mask during surgery. The area of surgery was prepared by shaving the rodent's head, administering a Puralube™ lubricant ointment in both eyes and mounting the animal in a stereotactic apparatus using non-traumatic ear bars. A micro-drill was used to drill two close holes in the skull where 1.5 µl (600 pmoles) endothelin-1 (Human and Porcine, EMD Chemicals) at a concentration of 400 pmoles/µl was injected into the cortex region underlying each hole at a depth of 2.0mm over the time course of 5 minutes. The incision site was then sutured and painted with antiseptic solution.

Voluntary Oral Drug Administration:

The drug vehicle was 4 grams of purchased Pillsbury® sugar cookie dough. The cookie dough was weighed, rounded into a ball and then a depression pushed into the ball with a finger. The individually-weighed drugs (Pharmaceutical grade crushed tablets or contents of capsules) were put into the depression in the dough ball and rim edges of the depression were brought together and sealed so that all the dry chemicals were enclosed in the dough ball. The dough ball was thoroughly mixed manually to incorporate all of the chemicals into the dough, and reformed into a ball. Each ball either contained no drugs (vehicle) or the 5 mg/kg Fluoxetine plus 1 mg/kg Simvastatin. The dough balls were presented to individually-housed rats in a glass petri dish around 4:00 pm each day and left in the cage until the next day.

Montoya Staircase Training:

Animals were trained to retrieve sucrose pellets from the stair wells for 15 minutes each day during the animals' dark cycle, for a total of 14 days. We used maple extract on the sucrose pellets to enhance training.

Statistical Analysis:

All statistical analysis in this poster used 2-Way Analysis of Variance (ANOVA).

Exclusions:

Any animal failing to completely ingest post-stroke daily medicine or vehicle for a total of 4 days was completely excluded from the study. Any animal failing to retrieve at least 9 pellets in each forepaw after Montoya Training was excluded from the Montoya analysis. Animals failing to display at least a 20% functional deficit compared to pre-stroke values at some time during post-stroke tests in Montoya Staircase were excluded from the study.

Figure 1: Voluntary Physical Rehabilitation

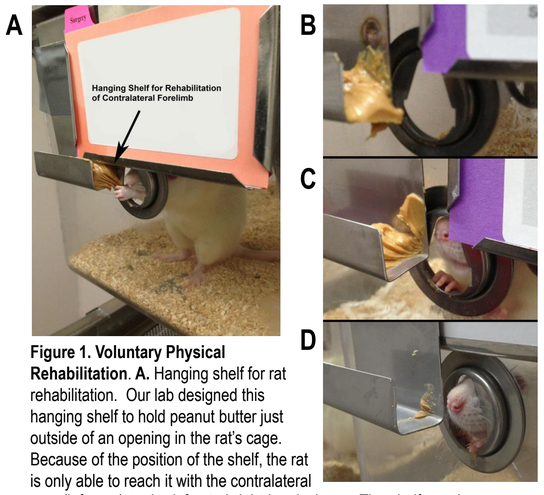

Figure 1. Voluntary Physical Rehabilitation. A. Hanging shelf for rat rehabilitation. Our lab designed this hanging shelf to hold peanut butter just outside of an opening in the rat's cage. Because of the position of the shelf, the rat is only able to reach it with the contralateral paw (left paw) to the infarcted right hemisphere. The shelf was hung every other night, beginning 8 days post-stroke and continuing for an overall period of 5.5 weeks. If animals refused to eat from the shelf, they were put into the No Rehabilitation group. All other animals generally began to eat from the shelves within three days. We could clearly determine if the animal underwent rehabilitation or not. In **B.** the animal has not eaten from the shelf, whereas in **C.** the animal has partially eaten from the shelf. In **D.** the animal has completely cleared the shelf of all peanut butter. We saw no difference in the number of completely-cleared versus partially-cleared shelves between the drug groups. This method enabled physical rehabilitation of the contralateral limb without the stress of physical restraint of the ipsilateral limb.

Group	Treatment	Rehabilitation	Duration of Treatment
1. (N= 14)	Vehicle Control	No	91 days
2. (N=14)	Vehicle Control	Yes	91 days
3. (N=14)	5 mg/kg Fluoxetine + 1mg/kg Simvastatin	No	91 days
4. (N=18)	5 mg/kg Fluoxetine + 1mg/kg Simvastatin	Yes	91 days

Table 1. Treatment Groups and Rehabilitation. In 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 Simvastatin, in Figure 3 animals from this study were compared with animals from a previous study which had received the drugs 20-26 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 rehabilitation, the hanging shelves were loaded with peanut butter and hung around 4:00 pm each day. The shelves were collected 24 hours later and evaluated for if the animal used its impaired paw. Animals which showed more than initial reluctance to use their impaired paw were put into the No Rehabilitation group. All other animals began using the shelves within three 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 rat. This hanging shelf rehabilitation accomplishes rehabilitation of the impaired limb without restraint, thus reducing stress during the rehabilitation.

Figure 2: Montoya Staircase (Assessment of Functional Recovery)

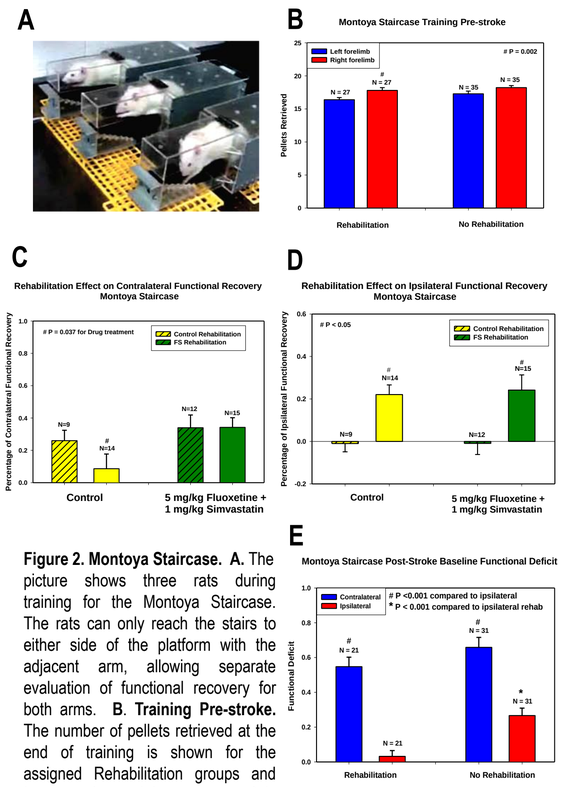

Figure 2. Montoya Staircase. A. The picture shows three rats during training for the Montoya Staircase. The rats can only reach the stairs to either side of the platform with the adjacent arm, allowing separate evaluation of functional recovery for both arms. **B. Training Pre-stroke.** The number of pellets retrieved at the end of training is shown for the assigned Rehabilitation groups and the Non-Rehabilitation groups. Rats retrieved from 16-18 pellets/forelimb by the end of training. There was a significant difference (P=0.002) between right and left forepaw performance in the rehabilitation group. **C. Rehabilitation Effect on Contralateral Functional Recovery.** The percentage of contralateral functional recovery (26%) in the Control Rehabilitation group (hatched yellow bar) is enhanced compared to the Non-Rehabilitation group (8%; solid yellow bar)(P=0.140). However, there was no significant difference in the percentage of contralateral functional recovery between the Fluoxetine & Simvastatin (FS) Rehabilitation group (hatched green bar) and the FS Non-Rehabilitation group (solid green bar), where both are showing 34% contralateral recovery (P= 0.98). There is a significant difference between drug-treated groups and control groups (P=0.037) and a significant difference between Non-Rehabilitation groups with drug treatment versus control (P=0.014). **D. Rehabilitation Effect on Ipsilateral Functional Recovery.** 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 bars, ~-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 function. The ipsilateral side baseline deficit (26%) of the Non-Rehabilitation group was found to be significantly different from the Rehabilitation group (3%) (P<0.001). This may have been caused by slight differences in the depth of endothelin-1 injection in the stroke induction surgeries, causing the infarct to extend into the corpus callosum.

Figure 3: Infarct Size and Recovery (No Rehabilitation)

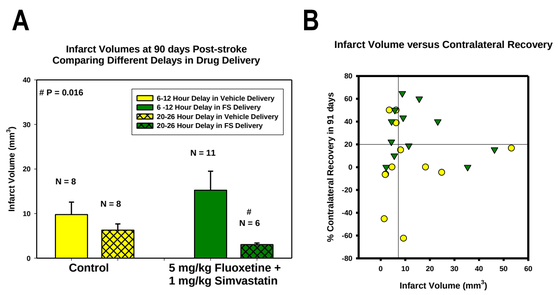

Figure 3: Infarct Size and Recovery in Animals with No Rehabilitation. A. Infarct volumes at post-stroke day 92 (solid bars) and post-stroke day 32 (hatched bars) are compared for animals that had same stroke induction procedure, but vary in the starting point of FS drug/vehicle delivery. We are comparing two different time points for the infarct volume, however in general the infarct volumes are known to decrease in size with time, as edema is reduced[2]. 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 drug delivery time is more beneficial when considering the final infarct volume. There is no significant difference in the control animal infarct volume based on when the vehicle is delivered. **B.** The individual infarct volumes from the animals who received the drugs (green triangles) or vehicle (yellow circles) 6-12 hours after stroke are shown along with the contralateral percentage recovery seen for that individual rat. Note that the optimal infarct volume for greater than 20% recovery with the drugs appears to be between 8 and 22 mm^3 in this group of animals. The very large infarct volumes associated with the drug treatment (greater than 30 mm^3) suggests that early drug delivery may result in secondary hemorrhagic stroke.

Conclusions

1. **The drug combination (Fluoxetine and Simvastatin) treatment achieved slightly greater motor functional recovery in rehabilitated animals compared to rehabilitated vehicle-treated animals.**
2. **Rehabilitation did not improve the functional recovery seen in the drug-treated animals.**
3. **Infarct volumes are significantly larger when the drug combination is given at an early time point (6-12 hours after stroke induction) versus a later time point (20-26 hours after stroke induction). Earlier treatment may lead to a secondary hemorrhagic stroke.**

References

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2. *Henrich-Noack, P, Baldauf, K, Reiser, G, and Reymann, K. Pattern of time-dependent reduction of histologically determined infarct volumes after focal ischaemia in mice. Neurosci. Letts. 2008; 432:141-156.*

Acknowledgements

- **Wright State University Graduate Student Assembly Original Work Grant to Moner A. Ragas**
- **Women in Science Giving Circle Grant to Adrian M. Corbett**
- **Internal Support from Jack Bantle, former Vice President of Research and Graduate Studies**