This study examined the effects of sonic whole body vibration on short-term memory formation and the production and death of neurons in the CA1 region of the hippocampus in cerebral hemorrhage-induced mice. Step-down avoidance test was performed to evaluate short-term memory. To examine neuron death, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL staining), indicative of DNA fragmentation, and immunohistochemistry of caspase-3, which appear in the terminal stage of protein degradation, were performed. To examine neurogenesis, immunohistochemistry for Ki-67 was performed.

1. Effect on short-term memory formation

Results of step-down avoidance test to evaluate short-term memory formation are shown in Figure 6. the control group took 283s, the hemorrhage-induced group 135s, treadmill running exercise group 249s, and 8Hz-, and 24Hz-vibration treated groups took 241s, 247s, and 234s, respectively.

Figure 6. Effect of sonic whole body vibration exercise on latency in step-down avoidance task.

(A) Sham-operation group,
(B) hemorrhage-induction group,
(C) hemorrhage-induction and treadmill running exercise group,
(D) hemorrhage-induction and vibration-8Hz exercise group,
(E) hemorrhage-induction and vibration-16Hz exercise group,
(F) hemorrhage-induction and vibration-24Hz exercise group.

Values are represented as the mean±SEM.
As can be seen from above, induction of hemorrhage reduced short-term memory formation in the CA1 region and this difference was statistically significant. Exercising on the treadmill or initiation of sonic whole body vibration exercise after hemorrhage induction enhanced memory formation. This observation was also statistically significant. However, there was no statistically significant difference among the different frequency exercise groups.

2. **Effect on neuron death**

(1) Change in the number of TUNEL-positive cells in the CA1 region

Figure 7 shows the TUNEL-positive cells in the CA1 region of the hippocampus, which indicates areas of neuronal cell death. The values for the control group, hemorrhage-induced group, treadmill exercise group, 8Hz-, 16Hz-, and 24Hz- vibration treated groups were 8.00±8.00/mm², 512.00±29.21/mm², 352.00±18.48/mm², 408.00±8.00/mm², 352.00±18.48/mm² and 400.00±20.66/mm², respectively.

Figure 7. Effect of sonic whole body vibration exercise on the number of TUNEL-positive cells in the CA1 region.

Upper: Photomicrographs of the TUNEL-positive cells. The scale bar represents 800 µm (1) and 100 µm (2)

Lower: The mean number of TUNEL-positive cells in each group.

(A) Sham-operation group,

(B) hemorrhage-induction group,

(C) hemorrhage-induction and treadmill running exercise group,

(D) hemorrhage-induction and whole body vibration-8Hz exercise group.

(E) hemorrhage-induction and whole body vibration-16Hz exercise group,

(F) hemorrhage-induction and whole body vibration-24Hz exercise group.

Values are represented as the mean±SEM.

As can be seen from above, brain hemorrhage increased the number of cells positively stained for TUNEL stain in the A1 region. Exercising on the treadmill or initiation of sonic whole body vibration exercise after hemorrhage induction reduced the number of TUNEL-positive cells. Both these observations were statistically significant. However, there was no statistically significant difference among the different frequency exercise groups.
As can be seen from above, hemorrhage increased the number of cells positively stained for caspase-3 in the CA1 region. Exercising on the treadmill or initiation of sonic whole body vibration exercise after hemorrhage induction reduced the number of caspase-3-positive cells. Both these observations were statistically significant. However, there was no statistically significant difference among the different frequency exercise groups.

(2) Change in the number of caspase-3-positive cells in the CA1 region

Figure 8 shows the caspase-3-positive cells in the CA1 region, which indicates dying neurons. The results for the control group, hemorrhage-induced group, treadmill exercise group, 8Hz-, 16Hz-, and 24Hz-vibration treated groups were 59.64±6.50/mm², 699.27±94.88/mm², 406.40±41.53/mm², 466.67±91.18/mm², 431.33±49.97/mm², and 483.20±115.08/mm², respectively.

Figure 8. Effect of sonic whole body vibration exercise on the number of caspase-3-positive cells in the CA1 region.

Upper: Photomicrographs of the caspase-3-positive cells. The scale bar represents 800 µm (1) and 100 µm (2).

Lower: The mean number of caspase-3-positive cells in each group.

(A) Sham-operation group,
(B) hemorrhage-induction group,
(C) hemorrhage-induction and treadmill running exercise group,
(D) hemorrhage-induction and vibration-8Hz exercise group,
(E) hemorrhage-induction and vibration-16Hz exercise group,
(F) hemorrhage-induction and vibration-24Hz exercise group.

Values are represented as the mean ± SEM.

As can be seen from above, hemorrhage increased the number of cells positively stained for caspase-3 in the CA1 region. Exercising on the treadmill or initiation of sonic whole body vibration exercise after hemorrhage induction reduced the number of caspase-3-positive cells. Both these observations were statistically significant. However, there was no statistically significant difference among the different frequency exercise groups.
3. Effect on neurogenesis

(1) Change in number of Ki-67-positive cells in the CA1 region

Figure 9 shows the Ki-67-positive cells in the CA1 region, which indicates neurogenesis. The results for the control group, hemorrhage-induced group, treadmill exercise group, 8Hz-, 16Hz-, and 24Hz-vibration treated groups were 272.00±58.86/mm², 1065.60±152.65/mm², 750.54±68.41/mm², 802.91±56.17/mm², 786.67±30.63/mm², and 817.45±45.79/mm², respectively.

Figure 9. Effect of sonic whole body vibration exercise on the number of Ki-67-positive cells in the CA1 region.

Upper: Photomicrographs of the Ki-67-positive cells. The scale bar represents 100 µm.

(1) Sham-operation group,

(2) hemorrhage-induction group,

(3) hemorrhage-induction and treadmill running exercise group,

(4) hemorrhage-induction and vibration-16Hz exercise group.

Lower: The mean number of Ki67-positive cells in each group.

(A) Sham-operation group,

(B) hemorrhage-induction group,

(C) hemorrhage-induction and treadmill running exercise group,

(D) hemorrhage-induction and vibration-8Hz exercise group.

(E) hemorrhage-induction and vibration-16Hz exercise group,

(F) hemorrhage-induction and vibration-24Hz exercise group.

Values are represented as the mean ± SEM.
As can be seen from above, brain hemorrhage increased the number of cells positively stained for Ki-67 in the CA1 region. Exercising on the treadmill or initiation of sonic whole body vibration exercise after hemorrhage induction reduced the number of Ki-67-positive cells. Both these observations were statistically significant. However, there was no statistically significant difference among the different frequency exercise groups.

4. Conclusion

This study examined the effects of sonic whole body vibration on short-term memory formation and the production and death of neurons in the CA1 region of the hippocampus in cerebral hemorrhage-induced mice. Through step-down avoidance tests, examination of TUNEL staining, immunohistochemical analyses of caspase-3, and Ki-67, the following conclusions were obtained:

1. Sonic whole body vibration in hemorrhage-induced mice enhanced short-term memory formation in a statistically significant manner.
2. Sonic whole body vibration in hemorrhage-induced mice inhibits an increase in neuron death due to hemorrhage in the CA1 region in a statistically significant manner.
3. Sonic whole body vibration in hemorrhage-induced mice inhibits an increase in neurogenesis due to hemorrhage in the CA1 region in a statistically significant manner.

The results in this study imply that Sonic whole body vibration in hemorrhagic patients can reduce brain damage due to neuronal death. Furthermore, it can play an effective therapeutic role in minimizing neurological disabilities caused by neuron death and accelerate the recovery process.

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