Biochemical Changes after Osteopathic Manipulative Treatment for Neuropathic Pain

Derek Tai[1], David Wang[1], Zhiyin Li[1], Gyunghwi Hannah Woo[1], Kevin Huang[1], Tony Huynh[1], Mark Santos, PhD[1], Germana Rappa, MD/PhD[1], Eric Toder, DO[1], Aurelio Lorico, MD/PhD[1]

[1]Touro University Nevada College of Osteopathic Medicine, Henderson, NV

Abstract

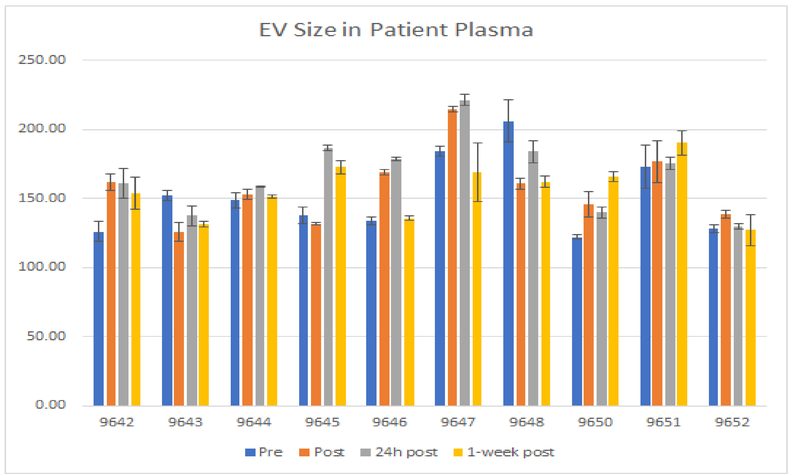
Neuropathic pain is a symptom that affects many people in their daily lives as a consequence of comorbid conditions such as obesity. The objective of this study was to analyze biochemical changes in patients with neuropathic pain before and after OMT. Specifically, we looked at beta-endorphin levels in extracellular vesicles (EVs) isolated from blood plasma at various time points and related the endorphin levels to the patient stress levels. Beta-endorphins reduce pain perception and we anticipated an increase in endorphins in response to OMT. We analyzed 10 patient plasma samples collected pre-OMT, directly after OMT, 24 hours after OMT, and 7 days after OMT. First, we examined whether OMT treatment resulted in changes in blood plasma EV size. We observed an increase in EV size after OMT with a gradual return to pre-OMT values 7 days later. Then, we examined by immunoblotting whether OMT treatment resulted in changes in blood plasma EV beta-endorphin levels. Normalized initial beta-endorphin values for 3 patient samples were 1.18, 1.3, and 5.45 which increased to 4.04, 1.52, and 11.04 respectively post-OMT. They showed gradual return to baseline values 7 days later. In conclusion, OMT caused an increase in blood plasma beta-endorphin levels, associated with an increase in EV size. These observations indicate that OMT can reduce physiologic stress on patients through increased production of endorphins, reducing pain. This information provides biochemical evidence of the efficacy of OMT and opportunities to study additional biochemical markers of OMT efficacy in greater detail.

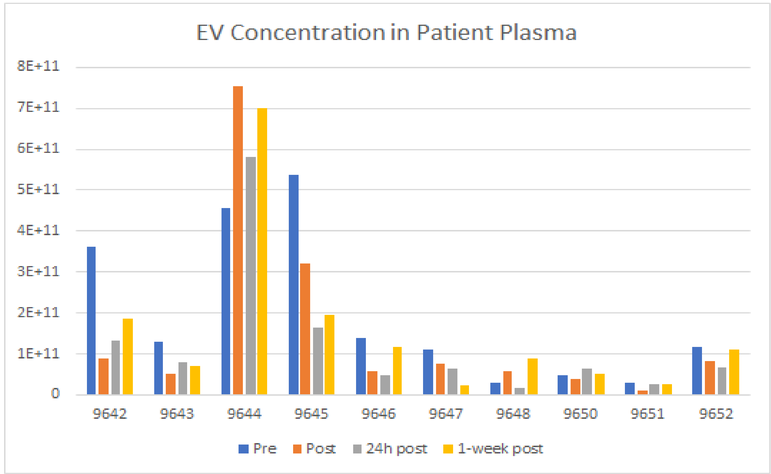
Study Design and Methods

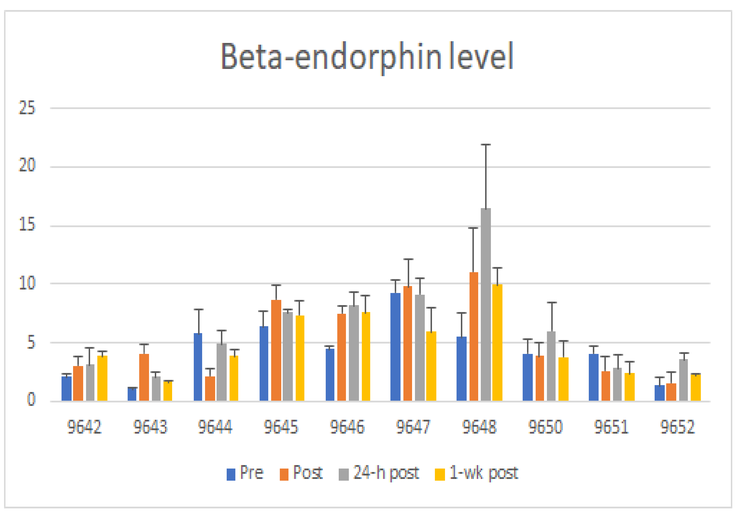
The same combination of myofascial release and balance ligamentous tension release techniques were utilized on all 10 patients. We then analyzed 10 patient plasma samples collected pre-OMT, directly after OMT, 24 hours after OMT, and 7 days after OMT. First, we measured changes in blood plasma EV size. The EV size was measured with Particle Metrix ZetaView NTA, calibrated and focus aligned with a 5% PBS solution. Each sample was measured 3 times to ensure accuracy and precision. Second, we used immunoblotting, specifically Western Blot, to measure changes in blood plasma EV beta-endorphin levels via ThermoFisher's Beta Endorphin Recombinant Rabbit Monoclonal Antibody. When antibody staining was completed, we used iBright FL1000 to image the immunoblot membrane. After, we used iBright Analysis to measure the concentration of beta-endorphin levels within the blood plasma EVs. The mean and standard deviation for each patient plasma sample are presented for pre-OMT, directly after OMT, 24 hours after OMT, and 7 days after OMT. Additionally, Patients took a pain scale survey for pre-OMT, 24-hr post OMT and 1-week post OMT.

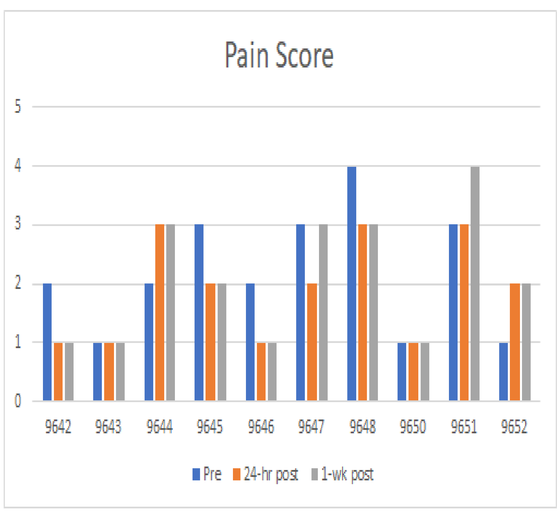
Results

Overall averages for EV size, concentration and beta-endorphin levels

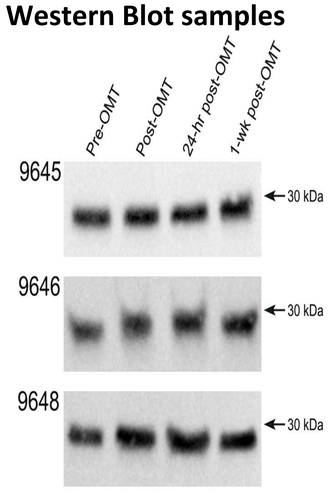








Western Blot samples



Conclusion

Our results suggest that there is a significant difference in EV size and beta-endorphin values post-OMT compared to pre-OMT values, demonstrating that OMT induces biochemical changes and physiologic reduction in pain in patients. Although we were able to only analyze 10 patient samples, these results provide many more opportunities to investigate the efficacy of OMT not only in neuropathic pain, but in other conditions like edema or vascular restrictions. We hope to recruit more patients in future studies and refine and expand our pain surveys to explore other biochemical changes during OMT and strengthen and validate the efficacy of OMT as an additional treatment option in clinical practice.

References

Properzi, F., Logozzi, M., and Fais, S. (2013) Exosomes: the future of biomarkers in medicine. *Biomark Med* 7, 769-778

Gardiner, C., Harrison, P., Belting, M., Boing, A., Campello, E., Carter, B. S., Collier, M. E., Coumans, F., Ettelaie, C., van Es, N., Hochberg, F. H., Mackman, N., Rennert, R. C., Thaler, J., Rak, J., and Nieuwland, R. (2015) Extracellular vesicles, tissue factor, cancer and thrombosis - discussion themes of the ISEV 2014 Educational Day. *J Extracell Vesicles* 4, 26901

Crewe, C., Joffin, N., Rutkowski, J. M., Kim, M., Zhang, F., Towler, D. A., Gordillo, R., and Scherer, P. E. (2018) An Endothelial-to-Adipocyte Extracellular Vesicle Axis Governed by Metabolic State. *Cell* 175, 695-708 e613