Concentration of Neural Regulators in Platelet Rich Plasma

Matthew Swift, Jesse Greene, Zachary Welch, Jennifer Woodell-May
Biomet Biologics, Inc., Warsaw, IN

Statement of Purpose: The use of autologous platelet rich plasma (PRP) is emerging in a variety of clinical modalities. Although the desired effects of PRP application vary across indications, the common mechanism of action is the up-regulation of the healing response at the site of application. This effect is most often attributed to the release of a broad array of cytokines from the alpha-granules during platelet degranulation. Research has demonstrated elevated concentrations of a variety of cytokines in PRP samples when compared to corresponding baseline blood samples. To date, no quantification of the concentration of neural regulators agents present in activated PRP samples has been reported. A number of neural regulators have been reported to be present in the alpha and dense granules of platelets with release occurring upon platelet degranulation. Upon release, these molecules elicit actions related to the hemostatic, inflammatory, and reparative processes associated with the wound healing processes. The current study measured the concentration of a variety of neural regulators in serum obtained from thrombin-activated PRP samples and compared it to corresponding serum obtained from whole blood samples.

Methods: A commercially available platelet concentrator (GPS II System, Biomet Biologics, Inc) was used to prepare ~6ml of PRP from 60ml citrate anticoagulated blood samples obtained from 9 healthy human volunteers. PRP and whole blood samples from each subject were analyzed for cellular content using a hematology analyzer (Cell Dyn 3700, Abbot Laboratories) and then activated with a bovine thrombin/calcium chloride solution. Following a 10-minute incubation period, the activated samples were centrifuged for 5 minutes and the resultant serum was collected and assayed for serotonin, adrenaline, noradrenaline, dopamine, and brain derived neurotrophic factor (BDNF) using commercially available ELISA kits.

Results: Platelet concentration averaged $196x10^3$ platelets/µl in the baseline samples and $1230\ x10^3$ platelets/µl in the PRP samples, a 6.28 fold increase. Serotonin serum levels increased 4.48 fold (base=201.9ng/ml, PRP=904.6ng/ml), noradrenaline serum levels increased 3.02 fold (base=286ng/ml, PRP=863ng/ml), and BDNF serum levels increased 3.06 fold (base=3.49ng/ml, PRP=10.6ng/ml). All three of these findings were statistically significant (paired t-test, α=0.05). Serum levels of adrenaline increased 4.61 fold (base=0.72pg/ml, PRP=4.61pg/ml), however this finding was not statistically significant. This is likely due to variability associated with glandular release of adrenaline caused by the needle stick for the blood draw. Dopamine concentrations were not detected in any of the samples using an ELISA with a sensitivity of 100 pg/ml. Figure 1 presents the average concentration factors and standard errors (SE) for the platelet count data and the four detected neural regulators.

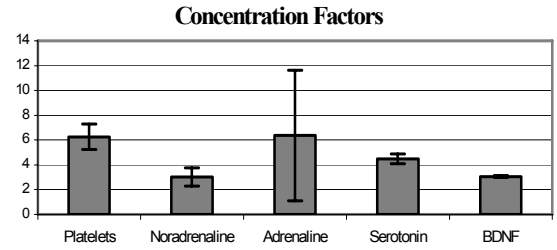

Figure 1-Average (±SE) concentration factor of platelets and detected neural regulators in activated PRP samples compared to corresponding base samples.

Conclusions: This study confirmed the presence of significantly elevated levels of serotonin, noradrenaline and BDNF in activated PRP samples compared to corresponding base samples. Further research designed to elucidate the role these elevated concentrations may contribute in the healing processes during clinical application of PRP would be of interest.