Viability and Differentiation of BMP-2 on W-20-17 cells in the Presence of Vancomycin.

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Statement of Purpose

As the population increases, bone injuries continue to rise and in one study infections were found in 65-70% of open musculoskeletal wounds.[1] Complications with infections and non-unions have led to the need for bone scaffolds which can aid in healing and bacterial abatement. Interactions of dual delivered pharmaceutical agents must be studied to ensure safety for patients. This study investigated the interaction of two pharmaceuticals used in bone fracture healing[2,3], a growth factor, Bone Morphogenetic Protein-2 (BMP-2) and an antibiotic, Vancomycin. The goal of this study was to determine the viability and efficacy of BMP-2 when delivered in the presence of vancomycin on osteosarcoma like cells.

Methods

*Test Solutions:*A stock solution of vancomycin-HCL was made in Dulbecco's Modified Eagle's Medium (DMEM). BMP-2 was reconstituted to 0.1mg/mL in DI H_2O containing BSA (50µg/1µg BMP-2). Dilutions were made in DMEM to cover ranges measured from studies on release of agents from calcium sulfate-chitosan composites in previous work. (Table 1)

Table 1: Test solutions for cell viability and BMP-2/vancomycin interaction studies in DMEM medium.

Vancomycin Conc (µg/mL)	BMP-2 Conc (ng/mL)
3600	1000, 500, 100, 50, 10, 0
1800	1000, 500, 100, 50, 10, 0
450	1000, 500, 100, 50, 10, 0
112.5	1000, 500, 100, 50, 10, 0
0	1000, 500, 100, 50, 10, 0

*Cellular Viability and Differentiation :*Murine osteosarcoma cells, W-20-17 (ATCC # CRL-2623) were seeded into 96 well plates at $3x10^4$ cells/cm^2 in DMEM medium containing 10% Fetal Bovine Serum and 1% Antibiotic/Antimycotics ON at 37°C and 5% CO_2. One plate per assay was made. Cells were washed three times with phosphate buffered saline (PBS) and 200µL of test solutions (Table 1) were applied and incubated for 24 hrs at 37°C and 5% CO_2. Viability was measured with Promega Cell Titer 96 AQ_{ueous} One Reagent and was applied per assay directions: 20µL reagent per 100µL sample volume, 2 hr incubation at 37°C and spectrophotmetrically read at 490nm. Alkaline Phosphatase (ALP) activity was measured as an indication of cellular differentiation. Plates were rinsed three times with PBS, 80µL of sterile/RNA free H_2O was added and incubated at RT for 10 min. Two freeze thaw cycles were then performed and 20µL of 0.75M Alkaline buffer and 100µL of 5mM Phosphatase Substrate were added to the wells and incubated 1hr at 37ºC. 0.3M NaOH stop solution was then added (100µL) and read at 405nm.

*Statistics:*Two way ANOVA was performed at α=0.05 and differences were evaluated with SNK post hoc tests.

Results

Viability of W-20-17 cells in the presence of test solutions showed no statistical difference between dose levels of BMP-2 (0.578) or interactions between the two groups (p=0.984), however, some vancomycin concentrations did cause decreases in cell viability (p=0.04 to p<0.001).

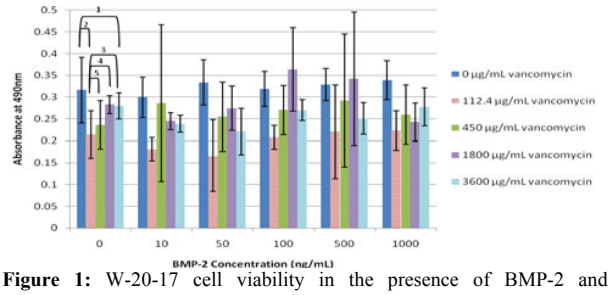

Figure 1: W-20-17 cell viability in the presence of BMP-2 and vancomycin (n=6). Numbers indicate statistical differences, 1) P=0.04, 2) P<0.001, 3)P =0.029, 4) P=0.003, 5) P=0.026 between vancomycin concentrations.

ALP expression was suppressed in cells exposed to higher levels of vancomycin and as BMP-2 levels increased, more statistical differences were seen between the groups. Statistical analysis indicated significant differences between BMP-2 (p< 0.001) and vancomycin dose level (p< 0.007) and interaction between the two (p< 0.001).

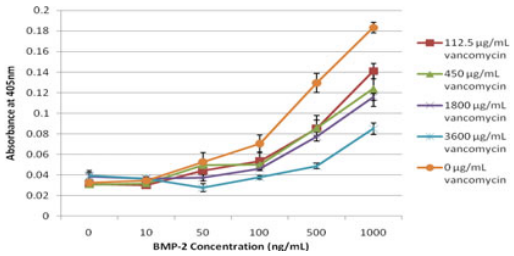

Figure 2: ALP production by W-20-17 cells in response to varying levels of BMP-2 in the presence of vancomycin (n=6).

Discussion/Conclusions

The range of vancomycin and BMP-2 tested was selected based on elution studies from bone scaffolds in the laboratory. While there was not an overt cytotoxic effect of the test solutions on the cells, all mock eluates that contained vancomycin did result in a suppression of ALP by the W-20-17 murine cells as compared to BMP-2 mock solutions that did not contain any vancomycin. This indicates that released vancomycin levels may interfere with BMP-2 stimulated osteogenesis.

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References

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