Effect of Heparin at Oxide Nitric Production and Metalloproteinase Activity on Pre-osteoblastic Cells

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INTRODUCTION: In a dynamics process, the formation and differentiation of bone tissue are related to factors present on extracellular matrix. During differentiation, osteoblasts synthesize a variety of molecules and compounds, like metalloproteinases(MMP), alkaline phosphatase(ALP) and nitric oxide(NO), which can be used as markers to osteoblastic differentiation. **OBJECTIVES:** Investigate the effect of heparin(Hep) on proliferation, differentiation, viability, calcium deposition, activity metalloproteinase and NO production at MC3T3-E1. **MATERIAL AND METHODS:** Hep was submitted to chemical analysis. For culture assays, MC3T3-E1 were incubated in presence or absence of ascorbic acid(AA, 40µM) or Hep(20-100µg/ml). MC3T3-E1 were maintained in culture for 1,3,5,7,9 days to cell proliferation assay by trypan blue and 1,3,5,7 days for viability assay by MTT. Cells incubated for 5 days with Hep or AA were analyzed to calcium deposition by Alizarin-Red, and for 7 days were analyzed to production of ALP. To NO dosage and metalloproteinase activity MC3T3-E1 were incubated in presence/absence of AA(50µM) or Hep(25,50,100µg/ml) **DISCUSSION AND RESULTS:** Hep is composed uronic acid(51%), hexosamine(36%) and sulfate(10%). Using trypan blue was observed that MC3T3-E1 in culture for 9 days showed a population doupling time(PDT) of the order of 1.71-3.51 as compared to initial number of cells plated(PDT 1.0). In relation to mitochondrial function the viability MC3T3-E1 incubated with Hep(20-100µg/ml) was ~95% for 1,3,5, days investigated. Cells showed morphological modification at first day after incubation to Hep and on the second day to AA(40µM) as compared to control. After 5 days in culture was observed an increase in calcium deposits on cells incubated with Hep(100µg/ml) as compared to control. In cells treated with Hep was observed an increase of NO production and ALP expression compared to control. And also was observed a modification in metalloproteinase activity. **CONCLUSION:** Our results suggest that production of oxide nitric and metalloproteinase activity in MC3T3-E1 were affected by heparin.

Palavra chave: MC3T3-E1, heparin, nitric oxide,
Patrocínio: UFJF and CNPq