Introduction:

Heterotopic ossifications result in functional deficits or even ankyloses. The pathophysiology of heterotopic ossification is not fully understood. It is expected that osteoblasts may play a role. A correlation is observed in the occurrence of heterotopic ossification in patients suffering from traumatic brain injury or polytraumatic patients undergoing long time mechanical ventilation. Increased serum levels of pro-inflammatory cytokines are detected. In addition, levels of anti-inflammatory cytokines increase during the course of the disease. This lead to the question whether cytokines influence osteoblastic activity and therefore may play a role in the pathogenesis of heterotopic ossification. Osteoblastic activity was measured by means of alkaline phosphatase (AP) activity.

Methods:

Osteoblasts were cultured out of resected bone samples (n=10). The bone specimens were collected under aseptic conditions and stored in 0.9% saline until preparation. All specimens were cleaned of loosely adherent bone marrow by vigorously washing with 0.9% saline several times. This procedure also removed blood components. Bone specimens derived from the iliac crest were divided into 2x3 mm chips. To allow outgrowth of osteoblasts from the bone tissue specimens, 20 chips of each sample were transferred into Petri dishes. Outgrowth of osteoblasts is only possible, when the bone samples have stable contact with the bottom of the petri dish. In order to fulfil this criterion fibrin glue was used. The fibrin glue allows firm adhesion of the bone samples to the bottom of the petri dish. BGJb Medium was added to the bone samples as a culture medium. The medium was supplemented with 10% foetal calf serum (not heat inactivated), 10 mM β-glycerolphosphate, and 50 µg/ml ascorbic acid. Amphotericin B and Gentamycin were added as antibiotics. Cells were allowed to grow out of bone explants and reach confluence. A number of 2.5x10^5 osteoblasts from the third subculture were plated in 6-well dishes. The osteoblasts were stimulated with 10 pg/ml of TNFα, IL-1β, IL-6, or IL-10 for 4 hours. As a negative control, no stimulating agents were applied to the cells. AP activity was determined in the culture supernatant and in the cell homogenate by addition of p-nitrophenylphosphate. The enzymatic release of the phosphate group results in a decrease of extinction at 405nm. This decrease during 3 minutes corresponded to the AP activity. Osteocalcin was detected using ELISA technique.

Essential results:

The AP activity of the negative control was said to be 100%. Overall, the activity was more in the supernatants than in the cell homogenate. TNFα resulted in the highest AP activity in the supernatant (316%). The other cytokines (IL-1β, IL-6, and IL-10) resulted in activities of around 250%.

TNFα did not cause an increase of AP activity in cell homogenates (103%). IL-1β increased AP activity in cell homogenates of 203%. IL-6 and IL-10 stimulated AP activity to 140% in cell homogenates.

The concentration of osteocalcin did not differ between non-stimulated and cytokine-stimulated osteoblasts. This was determined both in supernatants and cell homogenates.

Discussion:

TNFα and IL-6 cause an increased secretion of AP, whereas IL-1β leads to an increase in both secretion and production. Both pro- and anti-inflammatory cytokines result in an increased AP activity of osteoblasts. AP is a marker of osteoblast activity. On the other hand, the differentiation status was not changed by the cytokines as the differentiation marker osteocalcin was not altered under cytokine stimulation. As the investigated cytokines are increased in polytraumatic patients, these mediators may play a role in the pathogenesis of heterotopic ossifications. This can be due to enhancing the osteoblastic activity with consolidating osteoblastic phenotype. Future experiments should test different cytokine concentrations and different time courses. This should be combined with determining the amount of extracellular matrix and the composition of it.