Indometacin and prostacycline: antagonists in osteoblastic differentiation?  
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INTRODUCTION  
Heterotopic ossifications (HO) can not be distinguished from orthopic bone formation and appear especially in younger patients after osteotomies/fractures and after spinal and cerebral injuries. The intrinsic pathomechanisms for HO are still poorly understood in detail. One important pathogenetic pathway in ectopic bone formation is the induction of cyclooxygenases-2 (COX-2) resulting in increased expression and levels of prostaglandins which are a potent stimulator of osteoblastic differentiation. In different clinical studies a significant reduction of HO after total hip replacement could be achieved by prophylactic perioperative administration of COX-2 inhibitors (i.e. meloxicam, parecoxib & indometacin) [1]. One on the other hand some data indicate an osteoblast-protective potential for the prostacyclin-analog iloprost, as shown in early stages of avascular osteonecrosis (AVN) [2].

The following in-vitro study investigates the effect of the COX-2 inhibitor indometacin on osteoblastic differentiation and the potential influence of iloprost.

METHODS  
Human bone marrow cells from three donors were harvested via vacuum aspiration of the iliac crest followed by density gradient centrifugation (informed consent). Mononuclear cells were cultivated and expanded until confluency was achieved. To control the mesenchymal nature of these cells flow cytometry against defined antigens was performed. CD44+, 73+, 90+ & CD105+ as well as CD14+, 34+ & CD45+. The cells of the 1st & 2nd passage were seeded with 5000 cell/cm² and stimulated as follows: group A: indometacin (Indo) 10−6M (1), 10−5M (2), 10−4M (3), group B: Indo 1-3 × 10−5M iloprost; cells without stimulation served as control. After 10 and 28 days in-vitro the cells were analyzed morphologically and immunocytochemically for osteoblastic differentiation markers collagen I, Runx2, ALP, osteocalcin (OC), neuretinin receptor (NK-R) CD105, CD34, CD45 and the osteohistoinhibitory marker Twist1. Here, a standardized semiquantitative evaluation system was used: no positive cells (+), < 10 positive cells (++), 10-50 positive cells (+++), > 50 positive cells (+++). In addition, osteoblastic gene expression was investigated by RT-PCR. Cell proliferation was controlled by LDH-assay.

RESULTS  
The expression of the osteoblastic markers Runx2 and ALP increased with cultivation time while on the other hand expression of Twist1 decreased continuously (Fig. 1, Table 1). Both hematopoetic markers (CD34, CD45) could not be detected. Osteocalcin expression was strong while collagen I was only weakly expressed. In iloprost stimulated cultures increased indometacin levels suppressed cell proliferation (Fig. 1; correlation coefficient = 0.98). The present in-vitro data indicate a dose-dependent reduction of cell proliferation and inhibition of osteoblastic differentiation of mononuclear bone marrow cells by indometacin. On the contrary the addition of iloprost did not significantly influence cell proliferation nor promoted the expression of Runx2, ALP and osteocalcin.

Figure 1. Expression of different osteogenic antigens after indometacin stimulation of the bone marrow cultures in 3 different concentrations.

DISCUSSION  
There is an ongoing controversial discussion about the pathogenetic meaning of prostacyclin and analogous substances for osteogenic differentiation of osteoblast progenitors. Because iloprost was applied successfully in the treatment of painful bone marrow and early stage AVN an pro-osteogenic effect was hypothesized [3,4]. In addition, it has been shown previously that prostacyclin (PGI2) is an important mediator implicated in bone metabolism which acts via the kinase A-pathway as a potent inhibitor of bone resorption and mediates bone modelling [5].

However, our data indicate that iloprost is not able to antagonize indometacin-related inhibitory effects in osteoblast differentiation of human bone marrow cells in-vitro. These data suggest that a reduction of bone marrow edema which can be noted in patients after iloprost treatment may be based more on the vasodilative effects than on a stimulation of osteoblastic differentiation.

REFERENCES  

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Table 1. Semiquantitative evaluation of antigen expression after indometacin application with / without iloprost in human bone marrow cells.

Figure 2. Indometacin showed a dose dependent suppression in cell proliferation in bone marrow cultures which were also supplemented by iloprost.