ACTIVATION OF JAK/STAT SIGNAL TRANSDUCTION PATHWAY BY ALENDRONATE: NOVEL MECHANISM OF ACTION?

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**Introduction:** Bisphosphonates (BP) are a family of analogous synthetic pyrophosphates whose function as suppressor of bone resorption has been known for several years. Alendronate (Al) is a 2nd generation bisphosphonate currently being used to treat bone loss associated with osteoporosis and Paget’s disease. Because of its inhibitory action on bone resorption, several clinical trials have been started to determine its therapeutic potential in periprosthetic osteolysis and aseptic implant loosening. Although alendronate has been shown to inhibit a rate-limiting step in the cholesterol biosynthesis pathway in osteoclasts [1, 2], its action on other intra-cellular targets in osteoclasts as well as in other cell types is not clearly understood [3]. The present study indicates that in PBMCs or in THP-1 cell line, alendronate can activate interleukin-6 (IL-6) synthesis, which in turn activates Janus kinase / signal transducer and activator of transcription (JAK/STAT) signal transduction pathway leading to transcription of genes whose products inhibit bone resorption. STATs are transcription factors that are present in latent form in the cytoplasm and are activated through phosphorylation by the action of the receptor associated Janus family of kinases (JAKs). The activated STATs then form dimers and translocate to the nucleus where they bind to STAT binding sequences in the cytokine inducible genes and promote transcription. Specific STATs are activated by specific cytokines, as for example, IL-6 activates Stat1 and Stat3, while interferon-gamma (IFN-γ) activates only Stat1 [4]. IL-6 has recently been shown to have negative regulatory effect on cytokine induced such as tumor necrosis factor-alpha, and Interleukin-1beta [5], that have known osteoclastogenic functions. Very recently, it was shown that TNF-α and IL-1β, but not IL-6, stimulated osteoprotegerin ligand (OPGL, also known as TRANCE, or RANKL, a major osteoclastogenic factor) gene expression in human osteoblastic cells [6]. Furthermore, IL-6 has been found to activate acute phase proteins like Alpha-2-Macroglobulin, which functions as a natural inhibitor of several serum proteases including cathepsin, chymotrypsin, trypsin, elastase, and trypsin, which play important roles in wear debris induced osteolysis and in joint tissue destruction in rheumatoid arthritis [7].

**Methods:** Human peripheral blood mononuclear cells (PBMCs), were incubated with alendronate (Fosamax, Merck, Inc, Rahway, NJ) at a concentration of 100 µg/ml for 18 hrs. As shown in Fig. 1, alendronate activated STAT DNA binding activity in PBMCs following 18 hours (overnight) of culture at 37°C and 5% CO2, while the activity went down almost to basal level after 2 days (42 hr) of culture. Absence of any STAT binding activity within 1 hour of treatment suggested indirect effect of alendronate on the cells, and was probably mediated through new protein synthesis. The DNA binding probe hSIE binds mostly Stat1 and Stat3, the same STATs activated by IL-6. The EMSA profile of alendronate activated STATs and IL-6 activated STATs looked very similar on the gel and both anti-Stat1 and anti-Stat3 antibodies ‘supershifted’ alendronate activated STAT complex (data not shown). To determine whether alendronate was modulating cytokine expression, we next incubated cells with alendronate in presence or absence of neutralizing antibodies against either IFN-γ or IL-6, for 18 hrs. As shown in Fig. 2A, IL-6 neutralizing antibody but not anti-IFN-γ antibody prevented alendronate mediated STAT activation in PBMCs. In order to further confirm the “active principle” in the culture media, we neutralized 18 hour alendronate treated PBMC culture supernatant with either anti-IL-6 or anti-IFN-γ antibody, then used the neutralized medium to treat THP-1 cells for 12 min, followed by extraction and EMSA. As shown in Fig. 2B, media from alendronate treated cells activated STAT activity in THP-1 cells, and the activating principle could be neutralized by anti-IL-6 antibody but not by anti-IFN-γ antibody.

**Conclusion:** Alendronate has been shown by this study to activate IL-6 biosynthesis in PBMCs, leading to activation of JAK/STAT signal transduction pathway in an autocrine fashion. The present study also indicates that alendronate may function by controlling synthesis of a cytokine which has anti-inflammatory activities. The present data will not only help us understand the mechanism of action of alendronate, but will also suggest ways to improve the efficacy of the drug by finding compounds that will work synergistically with it.

**References:**