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Mutual augmentation of the induction of the histamine-forming enzyme, histidine decarboxylase, between alendronate and immuno-stimulants (IL-1, TNF, and LPS), and its prevention by clodronate

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Abstract

Nitrogen-containing bisphosphonates (N-BPs), powerful anti-bone-resorptive drugs, have inflammatory side effects, while histamine is not only an inflammatory mediator, but also an immuno-modifier. In murine models, a single intraperitoneal injection of an N-BP induces various inflammatory reactions, including the induction of the histamine-forming enzyme histidine decarboxylase (HDC) in tissues important in immune responses (such as liver, lungs, spleen, and bone marrow). Lipopolysaccharide (LPS) and the proinflammatory cytokines IL-1 and TNF are also capable of inducing HDC. We reported previously that in mice, (i) the inflammatory actions of N-BPs depend on IL-1, (ii) N-BP pretreatment augments both LPS-stimulated IL-1 production and HDC induction, and (iii) the co-administration of clodronate (a non-N-BP) with an N-BP inhibits the latter's inflammatory actions (including HDC induction). Here, we add the new findings that (a) pretreatment with alendronate (a typical N-BP) augments both IL-1- and TNF-induced HDC elevations, (b) LPS pretreatment augments the alendronate-induced HDC elevation, (c) co-administration of clodronate with alendronate abolishes these augmentations, (d) alendronate does not induce HDC in IL-1-deficient mice even if they are pretreated with LPS, and (e) alendronate increases IL-1β in all tissues tested, but not in the serum. These results suggest that (1) there are mutual augmentations between alendronate and immuno-stimulants (IL-1, TNF, and LPS) in HDC induction, (2) tissue IL-1β is important in alendronate-stimulated HDC induction, and (3) combination use of clodronate may have the potential to reduce the inflammatory effects of alendronate (we previously found that clodronate, conveniently, does not inhibit the anti-bone-resorptive activity of alendronate).

Keywords: Bisphosphonates; Histidine decarboxylase; Lipopolysaccharide (LPS); Interleukin-1 (IL-1); Clodronate; Alendronate

Introduction

Among the bisphosphonates (BPs), many nitrogen-containing bisphosphonates (N-BPs, including the aminobisphosphonates) have bone resorption inhibitory activities that are much stronger than those of non-N-BPs (Geddes et al., 1994; Rodan and Fleisch, 1996; Rogers et al., 2000). These drugs are important against diseases involving an enhanced bone resorption (osteoporosis, tumoral osteolysis, tumoral hypercalcemia, osteogenesis imperfecta, Paget disease, and rheumatoid arthritis). The anti-tumor activity of N-BPs may also be clinically useful against bone-metastatic tumors (Lipton, 2004). However, most N-BPs have undesirable inflammatory side effects, such as fever, increase in acute-phase proteins, gastrointestinal disturbance, and ophthalmic inflammation (Adami et al., 1987; Siris, 1993; Macarol and Frauenfelder, 1994; Sauty et al., 1996; Fleisch, 1997; Thiébaud et al., 1997). Although non-steroidal anti-inflammatory drugs (NSAIDs) are currently used for treating the fever (Rauch and Glorieux, 2004; Robinson et al., 2004), NSAIDs themselves have ulcerogenic side effects. Moreover, N-BP treatment can lead

Abbreviations: BP, bisphosphonates; HDC, histidine decarboxylase; IL-1, interleukin 1; KO, knockout; LPS, lipopolysaccharide; N-BP, nitrogen-containing bisphosphonate; NSAID, non-steroidal anti-inflammatory drug; TNF, tumor necrosis factor.

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to jaw osteonecrosis (Ruggiero et al., 2004; Bagan et al., 2005) and, in children with osteogenesis imperfecta, to a potentially serious influenza-like reaction (Munns et al., 2004). Thus, development of a safe method to prevent their inflammatory side effects might increase the scope for applying N-BPs.

In murine-model experiments, a single intraperitoneal injection of an N-BP induces a variety of inflammatory reactions [including a prolonged induction of the histamine-forming enzyme, histidine decarboxylase (HDC)] together with changes in hematopoiesis (Endo et al., 1993; Nakamura et al., 1999). In such experiments, N-BPs induce a dose-dependent elevation of HDC activity at 5–40 μmol/kg (1.5–12 mg/kg) (Endo et al., 1993). These doses are larger than those used in clinical trials in terms of mg/kg. However, as described above, inflammatory reactions occur in many patients treated with intravenous N-BPs and also in significant numbers of patients treated with lower doses of oral N-BPs (Harinck et al., 1987; Schweitzer et al., 1995; Fleisch, 1997; Ruggiero et al., 2004). This suggests that human patients may be very sensitive to the inflammatory actions of N-BPs, and/or that the inflammatory actions of N-BPs might be augmented under certain conditions. Most patients with bone disorders require prolonged treatment with N-BPs, and such patients are often suffering from, or may catch, infectious diseases during the course of the treatment. Since pretreatment with an N-BP augments HDC induction by lipopolysaccharide (LPS, a cell-surface constituent of gram negative bacteria) (Sugawara et al., 1998; Funayama et al., 2000; Yamaguchi et al., 2000), we supposed that (i) N-BP treatment might augment the inflammatory reactions that occur when a patient catches an infectious disease and (ii) an infection might augment the inflammatory actions of N-BPs.

IL-1 and TNF (endogenous pyrogens) are implicated in various diseases, including those of the central nervous system (Dinarello, 1996; MacEwan, 2002; Anisman et al., 2003; Chesnokova and Melmed, 2002; Leon, 2002; Wang and Shuaib, 2002). LPS is a potent stimulator of the production of these cytokines, and indeed, they largely or partly mediate the actions of LPS (Dinarello, 1984, 1996; Beutler and Cerami, 1989). In addition to N-BPs and LPS, IL-1 and TNF also induce HDC in mice (Endo, 1989; Endo et al., 1986, 1992). Although LPS-stimulated HDC induction is not reduced in the IL-1-deficient mouse, N-BPs induce neither HDC elevation nor other inflammatory reactions in this artificial mutant mouse (Yamaguchi et al., 2000), indicating that the inflammatory actions of N-BPs may depend entirely on IL-1. We previously found that pretreatment of mice with alendronate (a typical N-BP or aminobisphosphonate) markedly augments both the IL-1 production and HDC induction stimulated by LPS (strangely, however, alendronate itself did not increase IL-1 in the blood) (Sugawara et al., 1998). These findings led us to hypothesize that N-BPs might also augment the inflammatory actions of IL-1 and TNF, the production of which may be enhanced in various inflammatory or infectious diseases.

Combined administration of an N-BP and clodronate (a non-N-BP) to mice largely abrogates the inflammatory actions of all N-BPs tested (Endo et al., 1999). We recently found that this combination retains the strong anti-bone-resorptive activity of alendronate (Monma et al., 2004), and in support of this in vivo observation, clodronate does not inhibit (rather, it enhances) the anti-bone-resorptive activity exhibited by low concentrations of alendronate in vitro (Frith and Rogers, 2003). We hypothesized that co-administration of clodronate and an N-BP might thus represent a strategy for preventing or reducing the latter's inflammatory actions while preserving its powerful anti-bone-resorptive activity.

As described above, HDC is commonly induced by N-BPs, LPS, IL-1, and TNF, and histamine is recognized not only as an inflammatory mediator but also as a regulator of immune responses, including the Th1/Th2 balance and hematopoiesis (Schneider et al., 2002). Here, to help test the basis of the hypotheses described above, we examined (i) HDC induction by alendronate in LPS-pretreated mice, (ii) HDC induction by IL-1 or TNF in alendronate-pretreated mice, (iii) changes in the levels of IL-1α, IL-1β, and TNFα in the serum and tissues of mice given alendronate, and (iv) the effects of co-administration of alendronate and clodronate in these experiments.

Materials and methods

Mice. BALB/c female mice (6–7 weeks of age) were obtained from the facility for experimental animals in Tohoku University. Homozygous BALB/c mice deficient in both IL-1α and IL-1β (IL-1KO mice) were provided by Dr. Iwakura (Tokyo University). IL-1KO mice were established by back-crossing to BALB/c mice from original IL-1KO mice (Horai et al., 1998). These mice were bred in our laboratory. All experiments complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University.

Reagents. Alendronate, clodronate (both synthesized by ourselves; Endo et al., 1999), or a mixture of the two was dissolved in sterile saline, the pH of the solution being adjusted to 7 with NaOH. Human recombinant IL-1β (Ohtsuka Pharmaceutical Co., Tokushima, Japan) and TNFα (Dainippon Pharmaceutical Co., Osaka, Japan) were each dissolved in sterile saline. A lipopolysaccharide (LPS) from *Escherichia coli* O55:B5 prepared by Westphal's method was obtained from Difco Laboratories (Detroit, MI, USA) and dissolved in sterile saline. These reagents were injected intraperitoneally (i.p.) (0.1 ml/10 g body weight), except in one experiment in which LPS was injected intravenously (i.v.). RPMI 1640 solution, Triton X-100, 1 M HEPES solution, bovine serum albumin, and gentamicin sulfate solution were obtained from Wako (Osaka, Japan). Proteinase inhibitor cocktail was from Sigma (St. Louis, MO, USA). Experimental protocols are described in the text or in the legend to the figure relating to each experiment. Previous studies have shown that (i) the inflammatory effects of N-BPs and the suppressing effect of clodronate are dose-dependent, and (ii) alendronate induces nearly a maximum HDC induction at 40 μmol/kg, an effect that is largely abolished by co-administration of the same dose of clodronate (Endo et al., 1993, 1999). Consequently, we chose that dose for both alendronate and clodronate in the present study.

Assay of HDC activity. HDC activity was assayed as described previously (Endo et al., 1993, 1998). Briefly, mice were decapitated and the tissues rapidly removed and stored in a jar with dry ice. Each tissue sample (less than 250 mg), having been put into a cooled Teflon tube with phosphorylated cellulose and 2.5 ml of ice-cold 0.02 M phosphate buffer (pH 6.2) containing pyridoxal 5′-phosphate and dithiothreitol, was then homogenized. The supernatant obtained after centrifugation of the homogenate was used as the enzyme solution. The histamine in the tissues was bound to the phosphorylated cellulose and was removed almost completely from the enzyme solution by the centrifugation. Reaction mixture (1 ml) containing the enzyme solution was incubated at 37 °C for 3 h with histidine. After the enzyme reaction had been terminated by adding $HClO_4$, the histamine formed during the incubation was separated by chromatography on a small phosphorylated cellulose column, then quantified