Abstract. Objective: To investigate the effects of ibandronate, a novel aminobisphosphonate, on inflammation as well as leukocyte-endothelial cell interaction in mouse antigen-induced arthritis (AiA).

Material and Treatment: 36 Balb/c mice were subcutaneously injected with 160 μg/kg of ibandronate once per day beginning at day 7 until day 13 after induction of AiA.

Methods: The severity of arthritis was assessed by changes of the transverse knee joint diameter. For the intravital fluorescence microscopy measurements on day 14 after AiA induction, the patella tendon was partly resected to visualize the intraarticular synovial tissue of the knee joint. The number of rolling and adherent leukocytes as well as red blood cell (RBC) velocity and functional capillary density (FCD) were quantified in synovial microvessels. Furthermore, leukocyte infiltration in the synovium was determined in histological sections with an established score.

Results: Both fractions of rolling leukocytes (p = 0.016) as well as number of extravasated leukocytes (p = 0.004) were enhanced in control animals treated with ibandronate in comparison to animals which received saline. Arthritic animals with and without ibandronate treatment revealed an increased FCD (p = 0.006, p = 0.008), enhanced number of rolling (p = 0.002, p = 0.001) and adherent leukocytes (p = 0.009, p = 0.007) and greater swelling of the left knee joint (p = 0.002, p = 0.001) when compared to control animals. No significant differences between arthritic animals and arthritic animals treated with ibandronate were found in any of the parameters assessed including leukocyte adherence, FCD, histology, and knee joint swelling.

Conclusion: Ibandronate treatment of healthy mice was associated with an enhanced fraction of rolling leukocytes and increased numbers of extravasated leukocytes indicating a proinflammatory effect on the synovial microcirculation. In animals with a preexisting antigen-induced arthritis, however, ibandronate did not induce an exacerbation of joint inflammation and leukocyte adherence.

Key words: Ibandronate – Antigen-induced arthritis – Synovial microcirculation – Intravital microscopy – Bisphosphonates

Introduction

Bisphosphonates are potent inhibitors of bone resorption and widely used for the treatment of disorders characterized by excessive bone turnover or bone loss such as Paget’s disease, malignant hypercalcaemia, tumoral osteolysis and osteoporosis [1–3]. Periarticular and generalized bone loss also occurs in patients with rheumatoid arthritis (RA) as an effect of the disease itself and due to medications like glucocorticoids and methotrexate. Therefore, bisphosphonate treatment is suggested to be beneficial in many RA patients [4–7].

The bone resorbing capacity of bisphosphonates varies greatly among their different derivatives. Aminobisphosphonates, such as pamidronate (3-amino-1-hydroxypropylidene-1,1-bisphosphonate) or alendronate (4-amino-1-hydroxybutylidene-1,1- bisphosphonate) are derivatives that contain a primary amino group and have been shown to inhibit bone resorption much stronger than non-amino-bisphosphonates [8–10]. Ibandronate (1-hydroxy-3-[1-methylpentamino]-propylidene bisphosphonate) is a novel aminobisphosphonate and a derivative of pamidronate. The inhibitory actions of ibandronate on bone resorption are 10 to 100 times stronger than those of pamidronate [11].

Although bisphosphonates are generally well-tolerated compounds with only few clinically relevant side-effects, up to 60% of patients receiving their first infusion of pamidronate or alendronate exert an acute phase reaction with increase in body temperature, lymphocytopenia, shivers, and myalgia [12–14]. The mechanisms underlying these effects remain unclear, but many authors believe that increased levels of proinflammatory cytokines such as IL-1β, TNF-α or IL-6 play a major role in this process [12, 15–18]. Nakamura et al. [10] found that treatment with amino-bisphospho-
nates (pamidronate and alendronate) provoked an exacerbation of collagen-induced arthritis in mice. Subsequently, the authors concluded that amino-bisphosphonates could worsen a preexisting inflammatory disease, such as rheumatoid arthritis, in the clinical application.

Ibandronate has also been found to be capable to induce a febrile response [19], however, in a recent study only pamidronate but not ibandronate treatment was associated with an increased body temperature and decreased counts of circulating lymphocytes [20]. Sauty et al. [15] demonstrated that ibandronate induces the production of TNF-α in vitro, but they noted no febrile response and no increase in plasma levels of TNF-α or IL-6 in patients treated with ibandronate. Finally, Richards et al. [21] reported proinflammatory effects of ibandronate in vitro and in vivo.

Since there is controversy about the possible proinflammatory actions and side-effects of ibandronate treatment, the aim of this study was to investigate the effects of ibandronate on inflammation as well as on the leukocyte-endothelial cell interaction in an animal model of antigen-induced arthritis.

Material and methods

Animals

36 female Balb/C mice were obtained from Charles River Wiga, Sulzbach, Germany. The animals were housed in cages of five, allowed food (smiff, Soest, Germany) and water ad libitum and kept in the Institute for Surgical Research 1 week prior to their first immunization. They were randomly assigned to the four treatment groups, control (n = 7), control with ibandronate (n = 8), AiA with saline (n = 7) and AiA with ibandronate (n = 9). 5 mice had to be excluded due to complications during surgical preparation. All experiments were conducted according to the german guidelines of animal protection laws.

Ibandronate administration

Ibandronate was generously provided by Roche (Basel, Switzerland). Ibandronate was diluted in sterile phosphate buffered saline solution (PBS) at a concentration of 20 µg/ml for injection into the animals. 200 µl of this solution (4 µg; 160 µg/kg) [22–24] were administered daily subcutaneously starting on day 7 after arthritis induction until the intravital microscopic measurements were made on day 14 after AiA induction. Control animals received injections of equivalent volumes of vehicle (PBS).

The dose (160 µg/kg) of ibandronate was selected because it represents the only established dose in mice and has been proven to be effective in reducing bone disease development in murine models of breast cancer and human myeloma [22–24].

Antigen-induced arthritis

Arthritis was induced in mice according to the protocol of Brackertz et al. [25]. On days – 21 and – 14, mice were first immunized with a subcutaneous injection of 100 µg of methylated bovine serum albumin (mBSA) (Sigma, Deisenhofen, Germany), dissolved in 50 µl of saline. The mixture was supplemented with 50 µl of complete Freund’s adjuvant (CFA) (Sigma, Deisenhofen, Germany) and 2 mg/ml of heat-killed Mycobacterium tuberculosis strain H37RA (Difco, Augsburg, Germany). Additionally, intraperitoneal injections of 2 × 10⁶ heat-killed Bordetella pertussis (Institute of Microbiology, Berlin, Germany) on days – 21 and – 14 were performed. Finally, arthritis was induced on day 0 by injecting 100 µg of mBSA dissolved in 50 µl saline into the left knee joint. Control groups underwent the same procedure, but only received equivalent volumes of saline.

Clinical assessment

Joint swelling was determined by measuring the transverse diameter of the knee joint with caliper in units of 0.1 mm.

Surgical preparation

Intravital fluorescence microscopy was performed in the synovial microcirculation of the mouse knee joint. The method allows for intravital microscopic study of microhemodynamic parameters, including vessel diameter and red blood cell (RBC) velocity in the microvascular network of the synovial microcirculation consisting of arterioles, capillaries, and postcapillary venules as well as assessment of functional capillary density (FCD) and leukocyte-endothelial cell interactions [26].

For the surgical preparation, anesthesia was induced by inhalation of isoflurane 1.2% and a combination of O₂ and N₂O. The mean arterial blood pressure was determined by means of an arterial catheter inserted into the tail artery and connected to a pressure transducer. The microsurgical procedure was performed as described previously [26]. Animals were kept on a heating pad to stabilize body temperature, which was controlled by a rectal probe. The left limb was placed on a stage with the knee slightly flexed. Immobilization of the extremity was achieved by fixation with silicon. After a 1-cm incision distal to the patellar tendon, we performed a partial skin resection. The patellar tendon was carefully mobilized and partly resected. Then the intraarticular synovial tissue of the knee joint was visualized. After superfusion with 2 ml of sterile saline, a cover glass was placed on the knee capsule and the intravital microscope was directed onto the synovium [26]. Special care was taken to avoid any constraints on the preparation. The animals were euthanized with 10 mg of pentobarbital intravenously after intravital microscopy.

Experimental protocol

The microscopic set-up has been described in detail elsewhere [27]. A 20-fold water immersion objective was used to select 2–4 regions of interest in each animal. These 4 regions contained postcapillary venules or capillary areas for the measurement of FCD or both. To measure the leukocyte-endothelial cell interaction, the fluorescent marker rhodamine 6G (Sigma, Deisenhofen, Germany) was injected intravenously in a single bolus of 0.15 mg/kg immediately before the measurement. Tissue epilumination was achieved with a 150 Watt variable HBO mercury lamp in conjunction with Zeiss (Jena, Germany) filter set 15 (band pass (BP) 546/12, Farbteiler (FT) 580, long pass (LP) 590). The FITC measurements were made using a variable 12 V, 100 W halogen light source and the Zeiss filter set 09 (BP 450–490, FT 510, LP 520). Measurements of vessel diameter, venular RBC velocity and FCD were made after a bolus injection of the in vivo fluorescent plasma marker FITC-dextran (mol mass 150 kDa; 15 mg/kg body wt i.v.) (Sigma, Deisenhofen, Germany). The microscopic images were captured with a CLD camera and recorded on S-VHS videotape using both filter blocks consecutively. Data analysis was performed off-line using a computer-assisted microcirculation analysis system [28].

Microcirculatory parameters

From the Rhodamine-stained video images, the leukocyte-endothelial cell interactions were quantified. Leukocytes interacting with the endothelium were classified as rolling or adherent cells. Rolling leuko-