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Fluvastatin does not prevent the acute-phase response to intravenous zoledronic acid in post-menopausal women

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ABSTRACT

The acute-phase response (APR) to aminobisphosphonates is triggered by activation of γδ T cells, resulting in pro-inflammatory cytokine release. Statins prevent aminobisphosphonate-induced γδ T cell activation *in vitro*, raising the possibility that statins might prevent the APR *in vivo*. The objective of this study was to determine whether fluvastatin prevents the APR to zoledronic acid in post-menopausal women. A double-blind, randomised, placebo-controlled study was conducted in 60 healthy, post-menopausal, female volunteers (mean age 60.6 ± 4.0). Volunteers received 5 mg zoledronic acid by intravenous infusion, and either three times 40 mg fluvastatin (0 hr, 24 hr and 48 hr), 40 mg fluvastatin (0 hr) plus placebo (24 hr and 48 hr), or placebo (0 hr, 24 hr and 48 hr), orally. Post-infusion symptoms were assessed by questionnaire. Changes in γδ T cell levels, pro-inflammatory cytokines (TNFα, IFNγ, IL-6) and C-reactive protein (CRP) were measured in peripheral blood at various time-points post-infusion. Zoledronic acid administration triggered increased serum levels of TNFα, IFNγ, IL-6 and CRP in ≥70% of study volunteers, whilst characteristic APR symptoms were observed in >50% of participants. Zoledronic acid also induced a transient fall in circulating Vγ9Vδ2 T cell levels at 48 hr, consistent with Vγ9Vδ2 T cell activation. Concurrent fluvastatin administration did not prevent zoledronic acid-induced cytokine release, alter circulating Vγ9Vδ2 T cell levels, nor diminish the frequency or severity of APR symptoms. In conclusion, intravenous zoledronic acid induced pro-inflammatory cytokine release and APR symptoms in the majority of study participants, which was not prevented by co-administration of fluvastatin.

This article is part of a Special Issue entitled Bisphosphonates.

Introduction

Bisphosphonates (BPs) are currently the most common treatment for a variety of disorders characterised by excessive osteoclastic bone resorption, such as post-menopausal osteoporosis [1], Paget's disease of bone [2] and tumour-associated osteolysis [3]. A common side-effect of intravenous administration of nitrogen-containing BP (N-BP), such as pamidronate or zoledronate (ZOL), is the development of a transient flu-like syndrome called the acute-phase response (APR). An APR typically occurs in 10–50% of patients receiving their first infusion [4–6] and is characterised by symptoms such as pyrexia and musculoskeletal aches and pains. These symptoms are frequently associated with increased circulating levels of pro-inflammatory cytokines such as IFNγ, TNFα and IL-6 [5,7–9]. Whilst the exact molecular mechanism underlying this phenomenon is not fully understood, it is becoming clear that activation of a specific subset of T cells, so-called γδ T cells, plays a central role.

γδ T cells were first identified as possible initiators of the APR to N-BPs by Kunzmann et al., who observed marked increases in the number of circulating γδ T cells in PAM-treated multiple myeloma patients up to 28 days post-infusion, which correlated with the severity of the APR [10]. Subsequent studies revealed that N-BPs specifically activate the major subset of γδ T cells in peripheral blood, Vγ9Vδ2 T cells [11]. N-BP-induced activation of Vγ9Vδ2 T cells in peripheral blood mononuclear cell cultures *in vitro* results in production of IFNγ, TNFα and IL-6 [5,12,13], which closely mirrors cytokine production triggered by N-BP administration *in vivo*. This suggests that strategies to minimise or prevent Vγ9Vδ2 T cell activation by N-BPs *in vivo* may prevent the APR.

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We and others have previously shown that activation of Vγ9Vδ2 T cells by N-BPs occurs via an indirect mechanism [12,14], requiring intracellular uptake of N-BP to inhibit its molecular target, farnesyl diphosphate (FPP) synthase [15–18]. Recently, we proposed that peripheral blood monocytes play a crucial role in Vγ9Vδ2 T cell activation due to selective internalisation of the N-BP by highly endocytic $CD14^+$ monocytes [19]. We demonstrated that, following a clinically relevant pulse of ZOL, inhibition of FPP synthase in monocytes causes the accumulation of the enzyme substrates, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), both of which are agonists of the Vγ9Vδ2-T cell receptor (TCR) [20].

Consistent with a central role for IPP and DMAPP accumulation in triggering Vγ9Vδ2 T cell activation, we and others have previously shown that the stimulatory effects of N-BPs on Vγ9Vδ2 T cell activation and proliferation in peripheral blood mononuclear cell cultures can be prevented by simultaneous treatment with a statin [12–14]. These widely-used cholesterol-lowering drugs inhibit HMG-CoA reductase, an enzyme upstream of FPP synthase, and thus prevent N-BP-induced IPP/DMAPP accumulation. We therefore investigated in this study whether co-administration of a statin could prevent ZOL-induced cytokine release and flu-like symptoms of the APR in healthy post-menopausal women.

Materials and methods

Study population and design

All research was conducted at Aberdeen Royal Infirmary, Aberdeen, UK. Ethical approval for this study was granted by the Grampian Local Research Ethics Committee. A total of 61 healthy women aged 50–70, who were >12 months post-menopause and had a *T*-score ≥2.5 (i.e. non-osteoporotic), were recruited into the double-blind, placebo-controlled study. Participants were BP-naïve and had not received hormone replacement therapy within 6 months of inclusion. Subjects receiving statin therapy were not excluded, but their therapy was suspended for 7 days prior to the ZOL infusion, and recommenced 7 days post-infusion. One participant withdrew from the study post-randomisation but before treatment commenced, resulting in 60 study participants. Subjects were randomly assigned to 3 treatment groups ($n=20$). All study participants received a single 5 mg zoledronic acid intravenous infusion over 15 min. In addition, one group (designated 3xFLU) received three times 40 mg oral fluvastatin (FLU) (at 0 hr, 24 hr and 48 hr); a further group (designated FLU + PLAC) received 40 mg oral FLU (at 0 hr) plus oral placebo (at 24 hr and 48 hr); the final group (designated PLAC) received oral placebo (at 0 hr, 24 hr and 48 hr). The first dose of FLU or placebo was administered 30 min prior to infusion of ZOL, to allow peak plasma concentrations of FLU during the ZOL administration, in line with the pharmacokinetic properties of FLU [21]. Study participants were allowed to self-administer acetaminophen to treat symptoms such as headache and pyrexia as required, and were encouraged to increase their fluid intake for 24 hr following the infusion.

Measurement of pro-inflammatory cytokines, C-reactive protein, cholesterol, and type I collagen C-telopeptide

Serum samples were obtained at baseline (0 hr), and at 4 hr, 8 hr, 24 hr, 48 hr, 4 weeks, 8 weeks and 12 weeks after infusion of ZOL, and stored at −80 °C until analysis. Levels of pro-inflammatory cytokines were determined using Quantikine ELISA kits (IFNγ & IL-6) or a Quantikine Highly Sensitive ELISA kit (TNFα) (all R&D Systems). Serum Hi-Sensitivity C-reactive protein (CRP) was determined using a standard nephelometric immunoassay on a Behring nephelometer with Behring reagents. Total serum cholesterol was determined using a standard colorimetric method on a Siemens auto-analyser with Siemens reagents. Serum levels of type I collagen C-telopeptide (CTx) were measured using a β-Crosslaps/serum kit and an Elecsys 2010 Immunoassay Analyser (Roche).

Proportion of Vγ9Vδ2 T cells in the circulation

Peripheral blood samples were collected at baseline (0 hr), 48 hr, 4 weeks and 8 weeks, using EDTA as anti-coagulant. Peripheral blood mononuclear cells (PBMCs) were isolated by density-gradient separation using Lymphoprep reagent (Axis-Shield). The number of Vγ9Vδ2 T cells in peripheral blood, relative to the total T cell population, was then determined by immunostaining using anti-human Vδ2-FITC (Beckman Coulter) and anti-CD3-PerCP (BD Biosciences) antibodies, followed by flow cytometric analysis. Data acquisition and analysis was performed on a BD FACSCalibur flow cytometer using CellQuestPro software.

Assessment of acute-phase reaction symptoms

Symptoms characteristic of the APR were assessed in 6 categories (joint aches; muscle aches; bone aches and pains; nausea; headache; flu-like symptoms) by questionnaire using a 4-point scale (1 = no symptoms; 2 = mild; 3 = moderate; 4 = severe). Questionnaires were completed at 8 hr, 24 hr, 48 hr and 72 hr post-infusion of ZOL.

Statistical analysis

The study was designed with the primary end-point being the effectiveness of FLU to inhibit the APR based on follow-up measurements of CRP. In assessing the power of the study it was assumed that at least 50% of subjects would get a >2-fold rise in their peak CRP values (CRP rising from a mean of 10 ± 4 mg/dl at baseline to 30 ± 12 mg/dl) and that pre-treatment with FLU would prevent this elevation. Based on these two assumptions, and a drop-out rate of 10% during follow-up, we aimed to recruit 60 women (20 subjects per group), in order to give 90% power with an alpha of less than 0.05. All statistical analyses were performed using SPSS software and SAS v9.1 (SAS Institute). A fixed-effects repeated measures analysis was conducted to test for statistical differences between the three treatment groups. Where a significant interaction was found between treatment group and time, differences between the treatment groups at each time point were investigated separately. To adjust for multiple comparisons, $p<0.01$ was taken to be statistically significant. Measurements that were not normally distributed (CRP, TNFα, IFNγ, IL-6, Vγ9Vδ2 T cells and cholesterol) were log-transformed and geometric means (95% CI) were calculated from the model. Two patients were excluded from the analysis due to abnormal baseline values of CRP and TNFα, respectively.

Results and discussion

Patient information and determination of FLU treatment protocol

Sixty female volunteers completed the study. The mean age was 60.6 ± 4.0 years. All volunteers were post-menopausal and presented with a *T*-score of ≥2.5. The subject disposition is shown in Fig. 1.

The proposed timing and dosage of FLU for the study was determined based on our findings in an *in vitro* model of the APR to N-BPs (Suppl. Fig. 1) using IFNγ release as a measure of N-BP-induced γδ T cell activation in PBMC cultures [12]. This showed that treatment of PBMCs with 1 μM FLU for 2 hr (which mimics the C_{max} following a typical 40 mg dose [21]) was effective in preventing γδ T cell activation induced by a pulse of 1 μM ZOL for 2 hr (which mimics the C_{max} following a typical *iv* dose of ZOL [22]). FLU pre-treatment period (3 hr, 1 hr, 0 hr) did not influence the effectiveness of FLU in our *in vitro* assay system (Suppl. Fig. 1), indicating that FLU does not need to be present long before ZOL to prevent γδ T cell activation. For