Geraniol Rescues Inflammation in Cellular and Animal Models of Mevalonate Kinase Deficiency

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Abstract. Background/Aim: The inhibition of the mevalonate pathway through genetic defects such as mevalonate kinase deficiency (MKD) or pharmacological drugs such as aminobisphosphonates causes a shortage of intermediate compounds, in particular geranylgeranyl-pyrophosphate (GGPP), which is associated with the consequent augmented IL-1β release in monocytes. Considering that, due to its biochemical structure, isoprenoid geraniol enters the mevalonate pathway and may revert the genetic or pharmacological inhibition, the present study tested isoprenoid geraniol in cellular and animal MKD models obtained through the use of aminobisphosphonate pamidronate. Materials and Methods: The effect of natural isoprenoid geraniol on bacterial induced-inflammation was evaluated in a monocytic cell line (Raw 264.7) and in Balb/c mice treated with pamidronate. Results: Geraniol diminished the levels of inflammatory markers induced by pamidronate stimuli in vitro and in vivo. Conclusion: Geraniol may be proposed as a novel therapeutic approach for the orphan disease MKD, and may also be considered for the evaluation of possible inflammatory side-effects of aminobisphosphonates.

Aminobisphosphonates (N-BPs) act on farnesyl-pyrophosphate synthase (FPPS) and inhibit the mevalonate pathway (1, 2). N-BPs are well-established as drugs in the treatment of bone diseases, such as osteoporosis or bone metastasis, because of their power to inhibit osteoclast-mediated bone resorption (2). Several studies have also investigated the effects of N-BPs on rheumatoid arthritis (RA), where the bone disruption is mediated by inflammatory-processes, but with disappointing results (3). In clinical practice, N-BPs have been shown to cause inflammatory side-effects, such as fever, an increase in the production of acute-phase proteins and inflammatory cytokines (TNF-α, IL-6), gastrointestinal disturbance and ophthalmic inflammation (2-4). Moreover N-BPs have shown pro-inflammatory effects in cellular and mouse models of disregulation of biosynthesis of cholesterol. In human peripheral blood monocytes (PBMCs), alendronate (Ald) and pamidronate (Pam) increased IL-1β secretion (5, 6), whereas Pam induced nitric oxide (NO) production by inflammatory chondrocytes (7). Ald-treated Balb/c mice have been shown to be very susceptible to compounds such as inflammatory cytokines (8), bacterial lipopolysaccharide (LPS) (8) and muramyldipeptide (MDP) (9).

The pro-inflammatory mechanism of N-BPs has been explained by the recent findings about mevalonate kinase deficiency (MKD, OMIM #251170). MKD is a rare auto-inflammatory disease caused by mutations within the second enzyme of the mevalonate pathway (mevalonate kinase, MK/MVK) (10). The consequent shortage of the intermediate geranylgeranyl-pyrophosphate (GGPP) has been associated with high IL-1β release from monocytes and with the dramatic systemic inflammation observed in MKD patients (6, 11, 12). Recently, a mouse model of MKD was described (9), showing that the chemical inhibition of the mevalonate pathway, through the use of alendronate, leads to an inflammatory phenotype that was amplified by bacterial MDP.

Isoprenoids, such as geraniol (GOH), are able to rescue inflammation in an MKD mouse model (9) as well as in Ald-LPS-treated Raw 264.7 cell lines (13) and in human monocytes (5). In the present study, Pam was used instead of Ald both in a Raw 264.7 cell line and in Balb/c mice, to support previous results regarding the inflammatory effect of N-BPs (5, 9, 13) and to demonstrate, definitively, that geraniol may revert the chemical inhibition of the mevalonate pathway.

Materials and Methods

Reagents. Bacterial MDP, Pam, and LPS (E. coli-serotype 055:B5), were obtained from Sigma-Aldrich (St Louis, MO, USA). GOH was obtained from Euphar group s.r.l. (Piacenza, Italy).
Raw 264.7 cell culture. Raw 264.7 cells (murine monocyte/macrophage cell line) (Sigma-Aldrich, Milan, Italy) were cultured in DMEM supplemented with 10% foetal bovine serum (FBS) with 20/50/100 μM Pam for 20 hours and then with 10 μg/ml LPS for additional 24 hours. At the end of the incubation the supernatant cells were collected for IL-1β assay. In some samples, GOH (100 μM) was added together with Pam.

Animals. BALB/c male mice (Harlan, Udine, Italy), aged six to eight weeks and weighting between 25-30 g, were used in this study, which was carried out in accordance with Italian laws (Ministry of Health registration no 62/2000-B, October, 6 2000) and was approved by the Ethical Committee of the University of Trieste. The experimental design was previously described (9). Briefly, the mice were randomly divided in groups of six animals each: group 1, controls; group 2, Pam (6.5/13/26 mg/kg) on day 0; group 3, Pam (6.5/13/26 mg/kg) on day 0 and MDP 100 μg/kg on day 3; group 4, MDP 100 μg/kg on day 3; group 5, Pam and MDP as in group 3 plus GOH 250 mg/kg. All solutions were administered intraperitoneally. After two hours of MDP administration, blood was collected directly into test tubes following decapitation of the mice. Serum was recovered by centrifugation at 2000 × g at 4˚C and then stored at –80˚C until used.

Determination of IL-1β concentration. IL-1β concentration was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Thermo Scientific Mouse IL-1β ELISA Kit; Pierce Biotechnology Inc., Rockford, IL, USA). The experimental procedures were performed according to the manufacturer’s protocols and the amount of IL-1β was expressed as pg/ml of serum.

Determination of NO concentration. NO concentration was evaluated in duplicate on supernatant cells using Griess Reagent (107 μM±9.6 to 126.5 μM±5), as expected, due to the inhibitory action of the aminobisphosphonate on mevalonate pathway (Figure 2C). Furthermore, MDP produced a general decrease of serum cholesterol levels both alone and in combination with Pam (Figure 2C).

Geraniol is able to reduce Pam+LPS-induced IL-1β and NO production in Raw 264.7 cells. When the isoprenoid GOH (100 μM) was added to Pam+LPS-treated Raw 264.7 cells, it reduced the IL-1β secretion (206 μM±6.22 vs. 979.7 μM ±104.4, respectively; p<0.001) (Figure 3A) and the NO production (11.5 μM±1.13 vs. 37.4 μM±2.14, respectively; p<0.001) (Figure 3B) in a statistically significant way. The GOH effect on IL-1β was not as dramatic on LPS-induced (128.3±8.75 μM) or Pam-induced (18.7 μM ±4.3) production; the data regarding the NO production was similar (data not shown).
Geraniol reduced inflammation in Pam+MDP-treated Balb/c mice. Compared to a Pam injection (d0), in a single day (d1 or d0 or d1) or in different time courses (d-1+d0, d-1+d1, d0+d1, d-1+d0+d1), the dose of 250 mg/kg GOH, given on days -1, 0 and 1 (d-1, d0 and d1, respectively) significantly reduced the SAA and IL-1β serum levels in Pam+MDP-treated mice (Figure 4A, B). The GOH anti-inflammatory effect was noteworthy for all combinations including day -1

Figure 1. Cytokine (A) and NO (B) production in Raw264.7 cell line stimulated with 20/50/100 μM Pam and 10 μg/ml LPS. Pam+LPS was compared to Pam at different concentrations using one-way ANOVA and Dunnett’s post-test. Moreover, Pam+LPS value were compared to LPS alone, using one-way ANOVA and Dunnett’s post-test. Data are expressed as the mean±SEM (n=3 replicates). *p<0.05; **p<0.01; ***p<0.001; ns, not significant (p>0.05).

Figure 2. Inflammatory markers in mice treated with Pam 6.5/13/26 mg/kg on day 0 and MDP at 100 mg/kg on day 3. (A) IL-1β, (B) SAA and (C) cholesterol levels. Pam+MDP was compared with Pam alone using one-way ANOVA and Dunnett’s post-test. Data are expressed as mean±SEM (n=3 replicates). *p<0.05; ***p<0.001; ns, not significant (p>0.05).
when compared to untreated mice or to other combinations. The SAA levels dramatic decreased in the presence of GOH: 89.0±8.2 mg/ml (d-1); 114.6±15.2 mg/ml (d-1/0); 111.8±0.21 mg/ml (d-1/1); 103.2±11.22 mg/ml (d-1/0/1) vs. 177.1±32.5 mg/ml; Figure 4A). A similar effect was shown for IL-1β: 133.7±40.4 pg/ml (d-1); 170.9±39.1 pg/ml (d-1/0), 170.4±35.9 pg/ml (d-1/1), 133.8±11.8 pg/ml (d-1/0/1) vs. 347.5±103.9 (Figure 4B). In addition, GOH was able to revert the effect of Pam+MDP on serum cholesterol levels in all daily combinations especially when administered on day 1 (Figure 4C).

Discussion

The immune modulation exerted by Pam has not yet fully been understood (15). In vitro experiments have shown an anti-inflammatory effect of this N-BP (16, 17), as well as a pro-inflammatory one (4, 6). Moreover, contrasting results have been obtained when Pam was used for the treatment of different inflammatory or immunological diseases, such as RA (18) or systemic sclerosis (19). Some authors have proposed that these different findings may be due to a collateral action of Pam. They hypothesise that not only does Pam inhibit FPPS (20, 21), as all the other aminobisphosphonates do, but it may also act on other still unknown pathways (22, 23).

The results of the present study showed that Pam is able to increase the sensitivity to bacterial compounds both in the murine macrophagic cell line Raw 264.7 and in Balb/c mice. This effect was directly dependent on the inhibitory action of Pam on FPPS, as shown by the reduced levels of serum cholesterol. These findings were in agreement with previously published data about serum cholesterol modulation in alendronate-treated mice (9, 24), suggesting a similar effect of Pam on the mevalonate pathway and excluding its hypothesised alternative site of action (22, 23).

In the models used in the present study, the effect of Pam seemed not to depend on its concentration as suggested by van Beek et al. (21). Interestingly, the concentration used in this study, 100 μM, was the same as that used by several other authors to obtain a higher percentage of FPPS inhibition (20, 21). Even though it has been reported that 0.1 μM Pam reduces FPPS activity by about 60% (20), no inflammatory effect was observed below 20 μM in the present study (data not shown). The results of the present study are similar to those obtained by Mandey et al. on human PBMCs (6). The effect of Pam seems to be involved in the increase of the susceptibility to pro-inflammatory compounds such as MDP.

When considering the effect of Pam in the presence of MDP, the present results suggest that, as a consequence of an inflammatory stimulus, a consumption of mevalonate isoprenoids occurs. Under normal conditions, these isoprenoids are continuously replaced through the mevalonate pathway, allowing a good feedback control of inflammation and a sufficient availability of intermediates for the synthesis of cholesterol.

The isoprenoid geraniol was able to reverse the Pam effect because it is supposed to enter the mevalonate pathway downstream the FPPS, metabolised probably by geranyl diphosphate, and finally bypassing the biochemical block obtained with the N-BP. GOH had a similar effect when alendronate was used instead of Pam (5, 9, 13). Moreover, the timing of GOH necessary to revert the pro-
inflammatory action of aminobisphosphonate was the same for Pam (Figure 3) and alendronate (9). Especially when given the day before Pam, GOH was able to reduce inflammation in Pam-MDP-treated mice, suggesting that the presence of the isoprenoid inhibits the action of Pam, resulting in reduced susceptibility to the action of the MDP. This finding may, therefore, be the starting point to assess the effect of different drug treatments to develop specific therapies to prevent, alleviate or suppress the inflammatory component in MKD.

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References


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