Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 516 (2005) 282 – 289

www.elsevier.com/locate/ejphar

Anti-inflammatory and analgesic effects of atorvastatin in a rat model of adjuvant-induced arthritis

Michele M. Barsante[a], Ester Roffê[a], Celina M. Yokoro[b], Wagner L. Tafuri[c], Danielle G. Souza[b], Vanessa Pinho[a], Maria Salete De A. Castro[b], Mauro M. Teixeira[a,*]

[a]*Departamento de Bioquímica e Imunologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627-Pampulha, 31270-901, Belo Horizonte, Brazil*

[b]*Farmacologia, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627-Pampulha, 31270-901, Belo Horizonte, Brazil*

[c]*Patologia Geral, Instituto Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antonio Carlos, 6627-Pampulha, 31270-901, Belo Horizonte, Brazil*

Received 21 March 2005; accepted 5 May 2005

Abstract

Statins exert favorable effects on lipoprotein metabolism but may also possess anti-inflammatory effects. Here, we explored the effects of atorvastatin in a model of adjuvant-induced arthritis in rat. Oral treatment with atorvastatin (1–10 mg/kg) from days 10 to 15 after arthritis induction caused inhibition of the increase in paw volume. Maximal inhibition occurred at a dose of 10 mg/kg. At this dose, atorvastatin markedly ameliorated the histopathological findings of joints obtained from day 16 of arthritic animals. This was mirrored by an effective blockade of neutrophil influx, as assessed by the tissue myeloperoxidase levels. The concentrations of the cytokines interleukin-1β, interleukin-6 and tumor necrosis factor-α and the chemokines CCL5 and CCL2 were significantly decreased in arthritic rats treated with atorvastatin. In contrast, the levels of interleukin-10 were enhanced by the drug treatment. The drug also prevented the hypernociception observed in the inflamed joints. These data clearly illustrate the therapeutic potential of a statin-sensitive pathway in inflammatory arthritis.

Keywords: Arthritis; Statin; Inflammation; Nociception; Cytokine

1. Introduction

Statins represent a well-established class of drugs that effectively lower serum cholesterol levels and are widely prescribed for the treatment of hypercholesterolemia. Statins competitively inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, reducing the availability of L-mevalonate and cholesterol biosynthesis (Kwak et al., 2003, 2000; Weitz-Schimidt, 2002). In addition to its anti-hypercholesterolemic effects, several reports have now described the ability of statins to interfere with secretion of cytokines and with leukocyte function and migration. For example, statins decrease the secretion of pro-inflammatory cytokines IL-6 (interleukin-6) and IL-8 (interleukin-8) from macrophages and inhibit the release of the chemokine CCL2/MCP-1 (macrophage chemotactic protein-1) from these cells (Chen et al., 2000; Zelvyte et al., 2002). The molecular mechanisms subserving such anti-inflammatory and/or immunomodulatory activities remain unclear.

Recent studies have focused on the ability of statins to modulate chronic inflammatory diseases, such as multiple sclerosis (Youssef et al., 2002). In animal models of the latter condition, atorvastatin prevented or reverted chronic and relapsing paralysis and inhibited the secretion of cytokines IL-2, IL-12, TNF-α (tumor necrosis factor-α), and IFN-γ (interferon type I). Rheumatoid arthritis is another common chronic inflammatory disorder that is characterized by joint inflammation with concomitant destruction of cartilage and bone (Swell and Trentham, 1993). Several pro-inflammatory cytokines, such TNF-α and IL-1β, and chemokines, such as CCL2 and CCL5, are

* Corresponding author. Tel./fax: +55 31 3 499 2651.
E-mail address: mmtex@icb.ufmg.br (M.M. Teixeira).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.05.005

reported to play a role in the pathogenesis of rheumatoid arthritis (Maini and Taylor, 2000; Feldmann et al., 2001; Kaplan et al., 2002). Although there are reasonably good drugs used in the symptomatic relief of arthritis, such as non-steroidal anti-inflammatory drugs, current treatment is still not satisfactory to modify fundamental pathologic processes responsible for the chronic inflammation (Lane, 1997). Cytokine-based therapies have been used for the treatment of rheumatoid arthritis and found to be useful in preventing progression of chronic arthritis in groups of patients. However, the latter therapies are based on the use of exogenous proteins (such as antibodies), which are costly and with the inherent possibility of an immune response against the exogenous protein and need for systemic injection.

Simvastatin treatment has been reported to improve the course of collagen-induced arthritis (CIA) in mice (Leung et al., 2003). Simvastatin administered intraperitoneally at a dose of 40 mg/kg was shown to suppress arthritis whereas lower doses of the drug (10 or 20 mg/kg) had no significant effect. Consistently, a controlled clinical study showed for the first time a potential effect of atorvastatin treatment on human rheumatoid arthritis (McCarey et al., 2004). In the present study, we have used a model of adjuvant-induced arthritis in rats to examine the effects of the treatment with atorvastatin on disease development. The drug was administered after the first signs of disease development (day 10 after the injection of adjuvant) and the following parameters were examined: increase in paw volume, nociception, pathology, neutrophil migration, and local production of pro-inflammatory cytokines and chemokines. The choice of atorvastatin was based on previous studies demonstrating the effectiveness of this drug on other models of chronic inflammation in rats (Youssef et al., 2002) and in preliminary data demonstrating effectiveness in humans (McCarey et al., 2004).

2. Material and methods

2.1. Animals

Female Holtzman rats (140–170 g) were used throughout this study. Animals were kept in cages (maximum of six animals per cage) at a room with controlled temperature (26 °C) and on a 12-h light–dark cycle. Water and food were given ad libitum. The local animal ethics committee has approved all experimental procedures described below.

2.2. Induction of arthritis by adjuvant

Rats were injected subcutaneously with a single dose of 0.2 ml mineral oil–water emulsion (10:1, *v/v*) containing 400 μg of dried *Mycobacterium butyricum* into the dorsal root of the tail under ether anesthesia, as previously described (Tatsuo et al., 1994; Francischi et al., 1997, 2000), for the induction of arthritis. Control animals were those injected subcutaneously with a single dose of 0.2 ml mineral oil–water emulsion (10:1) without *M. butyricum* into the dorsal root of the tail. The time of adjuvant injection is referred to as day 0. All experiments were repeated at least three times.

2.3. Treatment of animal with atorvastatin

Atorvastatin (Liptor, Pfizer Inc.) was brought into suspension in phosphate-buffered saline (PBS). Atorvastatin (1–10 mg/kg) or PBS were administered via oral gavage and animals were treated for 6 days. Treatment was initiated on day 10 after arthritis induction when the first signs of joint inflammation and pain are usually noted (Tatsuo et al., 1994; Francischi et al., 1997) and maintained until day 15. On day 16, the animals were sacrificed for histopathological, biochemical and cytokines analysis.

2.4. Measurement of hypernociception

The method for measuring hypernociception has been previously described elsewhere (Capetola et al., 1980; Tatsuo et al., 1994; Francischi et al., 1997). Briefly, the response of control (naive) and arthritic rats to five flexions of the tarsotibial joints of both hind paws was tested daily for 16 days starting from day 0 maintained until day 16. The results are reported as the mean (±S.E.M.) arthritis nociception index. The index is calculated by evaluating the number of vocalizations obtained following five flexions of hind limb tarsotibial joints. The local animal ethics committee has approved the procedures described above.

2.5. Measurement of edema

Hind paw volume (milliliters, as an indicator of edema) was measured daily using an Ugo Basile hydroplethysmometer (model 7150) after the test for hyperalgesia and is reported as the mean±S.E.M. All measurements were obtained at the same time of the day.

2.6. Histopathological processing and analysis

Arthritic paws were collected 16 days after the induction of arthritis and fixed in 10% buffered formalin. The fragments were then treated with a 10% acidic nitric solution for decalcification, dehydrated, cleared and embedded in paraffin. Serial sagittal sections of the whole paw were cut (3–4 μm thick), stained with hematoxylin and eosin and examined for the degree of synovitis and bone destruction in a blinded manner by one of the authors (WT). The following parameters were assessed: edema, synovial inflammation, bone and cartilage erosion and accumulation of neutrophil. Animals then received a semi quantitative score raging from 0 to 3 (0=no erosion, 3=extensive erosion, edema, cellular infiltration and bone destruction) depending on the intensity of the findings observed. The joints of at least three animals were observed in each experimental group.

2.7. Measurement of cytokines

For the measurement of tissue cytokine level, the subcutaneous tissue of the right hind paw and surrounding the tarsotibial joints was removed and placed on phosphate-buffered saline containing 0.05% Tween 20, 0.1 mM phenylmethylsulphonyl fluoride, 0.1mM benzamethonium chloride, 10 mM EDTA and 20 kallikrein international units of aprotinin A. The tissue was homogenized,

centrifuged at 3000 ×*g* for 10 min and stored at −70 °C until further analysis. The levels of TNF-α, IL-1β, IL-6, IL-10, CCL5 and CCL2 were evaluated using sandwich enzyme-linked immunosorbent assay (ELISA). ELISA kits for TNF-α , IL-1β, IL-6 and IL-10 were a kind gift of Dr Steve Poole (National Institute for Biological Standards and Control, Potters Bar, UK) and antibody pairs for CCL5 (Pharmingen, San Diego, California) and CCL2 (Peprotech, Veracruz, Mexico) were obtained commercially and used according to the instructions supplied by the manufacturer.

2.8. Determination of myeloperoxidase activity

The extent of neutrophil accumulation in the hind paw was measured by assaying myeloperoxidase activity, as previously described (Matos et al., 1999). Using the condition described below, the methodology is very selective for the determination of neutrophil over macrophages (data not shown). Briefly, the left hind paw tissue was removed and snap-frozen in liquid nitrogen. Upon thawing, the tissue (0.1 g of tissue per 1.9 ml buffer) was homogenized in pH 4.7 buffer (0.1 M NaCl, 0.2 M $NaPO_4$, 0.015 M NaEDTA), centrifuged at 10,000 rpm for 10 min and the pellet was subjected to hypotonic lyses (1.5 ml of 0.2% NaCl solution followed 30 s later by addition of an equal volume of a solution containing NaCl 1.6% and glucose 5%). After further centrifugation, the pellet was resuspended in 0.05 M $NaPO_4$ buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB) and was re-homogenized. One-milliliter aliquots of the suspension were transferred into 1.5 ml Eppendorfs tubes followed by three freeze–thaw cycles using liquid nitrogen. The aliquots were then centrifuged for 15 min at 10,000 rpm, the pellet was resuspended to 1 ml and the samples of hind paw were assayed. Myeloperoxidase activity in the resuspended pellet was assayed by measuring the change in optical density at 450 nm using tetramethylbenzidine (1.6 mM) and H_2O_2 (0.5 mM). Results were expressed as "myeloperoxidase index" and were calculated by comparing the optical density of hind paw tissue supernatant with a standard curve of neutrophil (>95% purity) numbers.

2.9. Drugs and reagents

Phenylmethylsulphonyl fluoride, benzamethonium chloride, EDTA, Tween 20 and aprotinin A were from Sigma (St. Louis, USA). Atorvastatin (Liptor™) was produced by Pfizer Inc. and purchased at the university pharmacy.

2.10. Statistical analysis

Data are presented as the mean ± S.E.M. of the shown number of experiments. Results were analyzed using analysis of variance and Student–Newman–Keuls *post-hoc* test. *P* values smaller than 0.05 were considered significant.

3. Results

3.1. Effects of the treatment with atorvastatin on hind paw volume increase, leukocyte influx and tissue pathology

The course of arthritis in animals injected with adjuvant was followed daily by evaluating the volume of both hind paws. As seen in Fig. 1, from day 10 onwards, there was a continuous increase of paw volume. Histopathological analysis of the tarso-metatarsal joints of arthritic animals on day 16 showed pronounced inflammation characterized by a diffuse mononuclear cell infiltration into sub-synovial tissue with cartilage and bone destruction (Fig. 2B). There was also accumulation of neutrophils along the surface of the synovium and intense edema. Neutrophil accumulation was also assessed by measuring the tissue myeloperoxidase content. As seen in Fig. 3, there was a marked increase in tissue myeloperoxidase activity on day 16 after adjuvant injection.

To determine the effect of the treatment with atorvastatin on the development of arthritis, the drug was given via the oral route from the first day that arthritis became clinically detectable, i.e. on day 10 (Fig. 1). Oral treatment with atorvastatin caused an inhibition of the increase in paw volume and maximal inhibition occurred at a dose of 10 mg/kg —at this dose, there was little increase in paw volume over that found in control animals (Fig. 1). The dose of 10 mg/kg was used in all further experiments.

In agreement with the inhibition of paw volume, treatment with atorvastatin markedly ameliorated the histopathological findings of joints obtained from day 16 of arthritic animals (Fig. 2C). Indeed, there was little leukocyte infiltration and cartilage or bone destruction was abrogated in treated animals (Fig. 2C). Atorvastatin-treated animals had a lower degree of sub-synovial inflammation that was still above that found in non-arthritic animals (Fig. 2A). Similarly, the increase of myeloperoxidase activity in arthritic animal was virtually abrogated in rats treated with atorvastatin (Fig. 3).

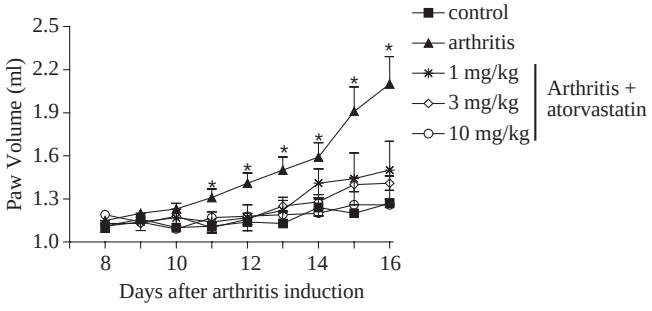

Fig. 1. Effects of the treatment with atorvastatin on hind paw edema in adjuvant-induced arthritis in rats. Adjuvant arthritis was induced and animals were treated with atorvastatin (1–10 mg/kg—closed symbols) via oral gavage or vehicle (PBS, open symbol) from days 10 to 15 after disease induction. Edema was measured using a hydroplethysmometer. Results are shown as the increase in paw volume (milliliters) and are mean ± S.E.M. for 5 animals in each group. Significant suppression of paw volume was observed in atorvastatin-treated rat when compared with untreated rats. The experiment shown is representative of three separate experiments. *$P<0.05$ when compared with control or atorvastatin rats.

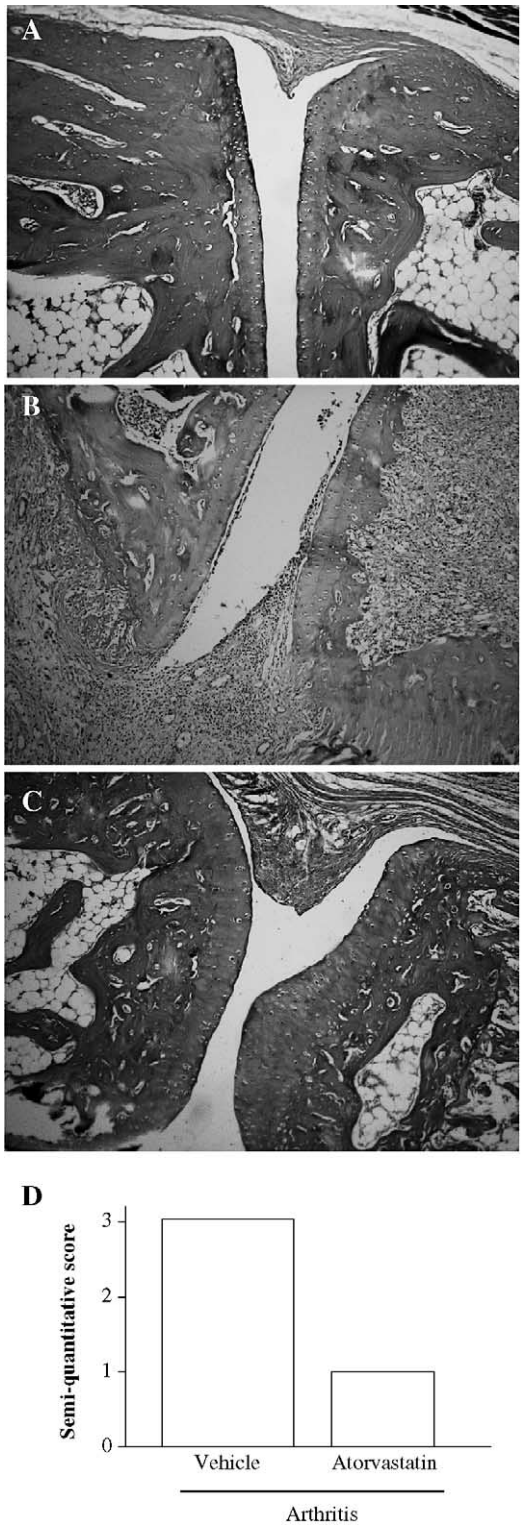

Fig. 2. Histological evaluation of the tarso-metatarsal joints. (A) Section of the joints of a naïve animal. (B) Section of a joint of an arthritic animal given PBS. Note the intense and diffuse inflammatory reaction with cartilage and bone erosion. The joint was obtained 16 days after the induction of arthritis. (C) Section of a joint of an atorvastatin-treated arthritic rat showing marked amelioration of pathological changes. (D) Semiquantitative analysis of the histopathological changes of the joints of arthritic rats. The scoring system is given in the methods section and results represent the median values of 3 animals in each group. Slides were stained with hematoxylin and eosin (× 400).

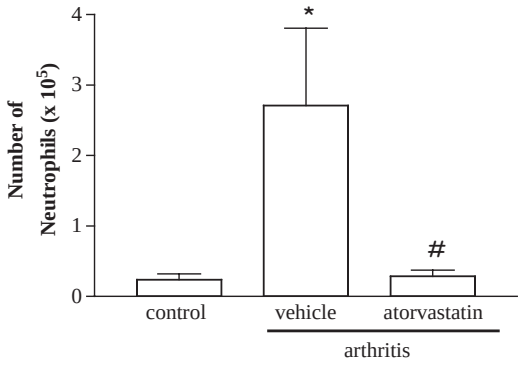

Fig. 3. Effects of the treatment with atorvastatin on the influx of neutrophils into the hind paw of arthritic rats. Adjuvant arthritis was induced and animals were treated with atorvastatin (1–10 mg/kg—closed symbols) via oral gavage or vehicle (PBS, open symbol) from days 10 to 15 after disease induction. Myeloperoxidase activity in the left hind paw was used as an index of neutrophil influx. Results are shown as the mean±S.E.M. of 6 animals in each group. *$P<0.01$ when compared with control rats and #$P<0.01$ when compared to arthritic rats.

3.2. Effects of the treatment with atorvastatin on the local production of cytokines and chemokines

There was a significant increase in the concentration of the proinflammatory cytokines IL-1β, TNF-α, and IL-6 and the chemokines, CCL2 and CCL5, in the hind paws of arthritic animals when compared to their controls (Fig. 4). Treatment with atorvastatin had a marked inhibitory effect on the local expression of all cytokines with 100%, 33%, 50%, and 100% inhibition of IL-1β, TNF-α, IL-6, CCL2 and CCL5, respectively (Fig. 4F).

To provide a greater insight into the mechanism by which atorvastatin treatment exerts its beneficial effect, we examined IL-10 cytokine expression, an anti-inflammatory cytokine that has been found to be protective in the pathogenesis of rheumatoid arthritis (Moore et al., 1993).The concentration of IL-10 in the hind paws was similar in arthritic and control animals, but there was a 37% increase in arthritic animals that received atorvastatin treatment (Fig. 4F).

3.3. Effect of the treatment with atorvastatin on hind paws hyperalgesia

Joint pain is the most incapacitating symptom in patients with arthritis. In arthritic rats, hypernociception was first noticed on day 9 after the injection of the adjuvant, peaked on day 11 and persisted till day 16 (Fig. 5). Treatment with atorvastatin starting on day 10 significantly prevented the hypernociception from day 11 onwards (Fig. 5).

4. Discussion

Statins are widely used for the prevention of cardiovascular disease. Although the beneficial effects of statins in preventing cardiovascular diseases may derive from their lipid-lowering activity, these drugs have also been shown to possess anti-inflammatory and immunomodulatory effects. These latter properties offer the potential for statins to

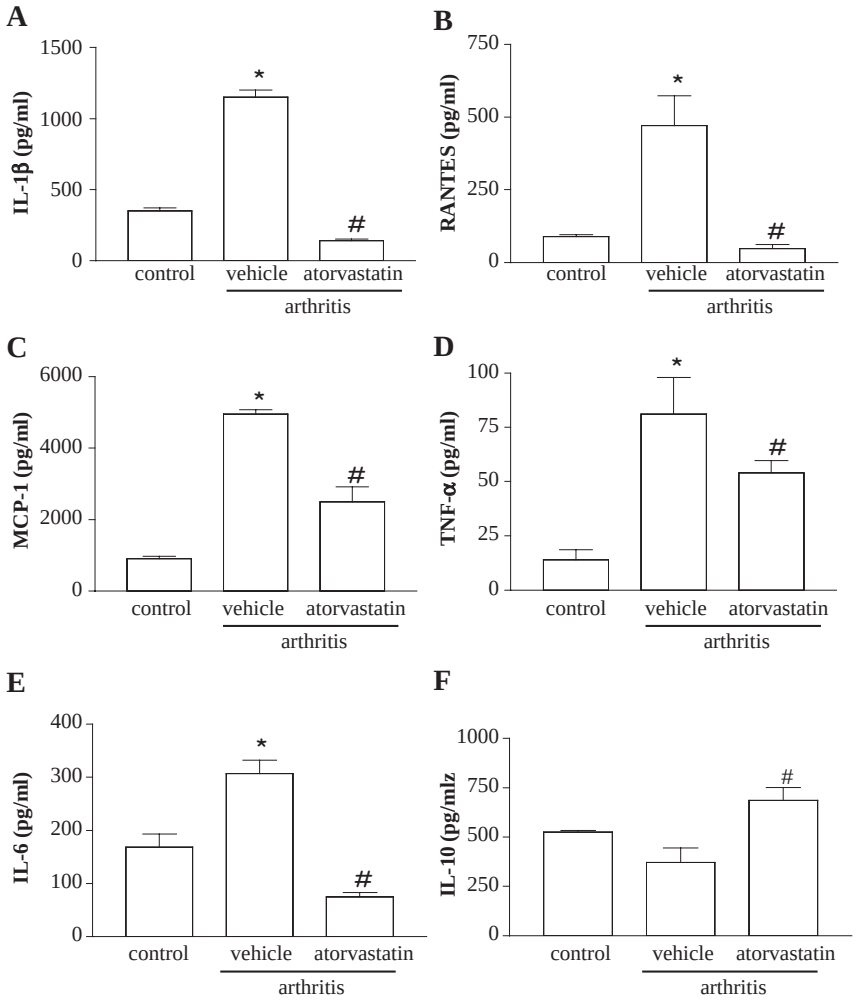

Fig. 4. Effects of the treatment with atorvastatin on the concentration of cytokines and chemokines in the hind paws of arthritic rats. Adjuvant arthritis was induced and animals were treated with atorvastatin (1–10 mg/kg—closed symbols) via oral gavage or vehicle (PBS, open symbol) from days 10 to 15 after disease induction. Twenty-four hours after the last administration of atorvastatin, the concentrations of IL-1β (A), TNF-α (B), CCL5 (C), CCL2 (D), IL-6 (E), and IL-10 (F) were assessed by ELISA on the supernatants of the homogenized tissues surrounding the right tarsotibial joints. Results are shown as the mean ± S.E.M. for five animals in each group. *$P<0.01$ when compared with control rats and #$P<0.01$ when compared to arthritic rats.

modify chronic inflammatory disease states. Indeed, several recent studies have demonstrated the ability of diverse statins to prevent chronic inflammation in vivo (Youssef et al., 2002; Jonhson et al., 2003; McKay et al., 2004) and at least one clinical trial has shown the beneficial effects of atorvastatin in patients with rheumatoid arthritis (McCarey et al., 2004). Here, we have evaluated the effects of atorvastatin in a model of adjuvant-induced arthritis in rats. Importantly, the administration of the drug was initiated after disease induction and just before the full development of joint inflammation. This model of arthritis resembles several aspects of human rheumatoid arthritis (Pearson, 1956) and appears to be useful for the development of potential analgesic and/or anti-inflammatory drugs (Colpaert et al., 1982).

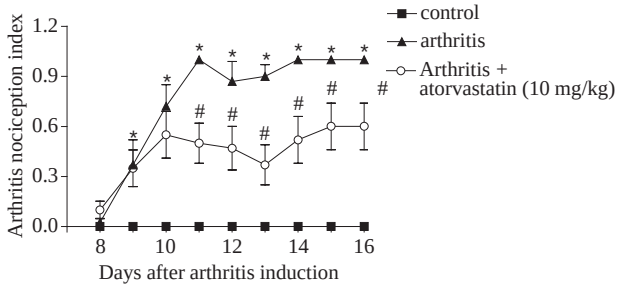

Fig. 5. Effects of the treatment with atorvastatin on the hind paw nociception index of arthritic rats. Adjuvant arthritis was induced and animals were treated with atorvastatin (1–10 mg/kg—closed symbols) via oral gavage or vehicle (PBS, open symbol) from days 10 to 15 after disease induction. Nociception was assessed and results are the mean ± S.E.M. of 5 animals in each group. *$P<0.01$ when compared with control rats and #$P<0.01$ when compared to arthritic rats.

The daily oral administration of atorvastatin effectively inhibited the increase of hind paw volume and inflammatory hypernociception. The inhibition of the increase in hind paw volume was associated with inhibition of neutrophil infiltration, as assessed by myeloperoxidase, and by significantly lesser tissue destruction, as assessed by histology. Our results also show that atorvastatin treatment

decreased the local production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and chemokines (CCL2 and CCL5). Only one other study has evaluated the effects of statins in animal models of arthritis. That study evaluated the effects of simvastatin in a model of collagen-induced arthritis in mice (Leung et al., 2003) and demonstrated significantly anti-inflammatory activity at a dose of 40 mg/kg. The latter study, however, did not investigate the potential effects of statins on chemokine production and inflammatory nociception.

The inhibition of hind paw neutrophil influx in rheumatoid arthritis may be a valid strategy to avoid tissue destruction (Bober et al., 2000). Neutrophils attach to the cartilage and invade the cartilaginous matrix and chondrocyte lacunae by releasing lysosomal enzymes and toxic radicals (Schalwijk et al., 1987; Ugai et al., 1983). In addition, neutrophils may be an alternative source of cytokines and chemokines and have the potential to participate in the cascade of events leading to further joint inflammation. In our study, there was marked inhibition of neutrophil influx, as quantified by myeloperoxidase activity and verified by tissue histology. This is consistent with the inhibition of neutrophil migration induced by statins treatment in other studies (Kanno et al., 1999; Okouchi et al., 2003). Thus, the observed inhibition of neutrophil influx to the joints of arthritic rats could contribute to the beneficial effects of atorvastatin in our system.

The pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 are shown to play an important role in the pathophysiology of arthritis development in animal models and in humans (Ivashkiv, 1996; Feldmann et al., 1996; Arend and Dayer, 1995). The effects of these cytokines in arthritic joints appear to be multiple and include the expression of adhesion and chemoattractant molecules, facilitation of leukocyte influx and activation (Cannnetti et al., 2003; Nanki et al., 2001; Liew and McInnes, 2002). For example, TNF-α blockade has been shown to be effective in experimental arthritis and in human disease (Joosten et al., 1996, Moreland et al., 1999). Similarly, IL-6-deficient mice do not develop bone erosion (Boe et al., 1999) and treatment with anti-IL-6 receptor antibodies may be effective in patients with rheumatoid arthritis (Nishimoto et al., 2003). Thus, blockade of the action of these cytokines, especially TNF-α blockade, is a valid anti-inflammatory principle in the treatment of arthritis. In our experiments, there was a marked blockade of IL-1β and IL-6 and a partial blockade of TNF-α production. It is not clear at present why TNF-α release was less inhibitable by atorvastatin treatment and whether this is relevant for the overall effects of the drug. Altogether, the latter results suggest that the ability of atorvastatin to inhibit pro-inflammatory cytokines may also contribute to the beneficial effects of the drug on this experimental model of chronic arthritis in rats.

It has been suggested that the anti-inflammatory cytokine IL-10 is an important factor in resolving chronic inflammation (Moore et al., 1993; Van Roon et al., 2001). A trend towards clinical improvement has been suggested in rheumatoid patients treated with recombinant human IL-10 (Katsikis et al., 1994). We found an increase in the concentration of IL-10 in arthritic animals given atorvastatin. Our experiments cannot suggest whether this increased production of IL-10 was relevant for the beneficial effects of the drug in our system. Nevertheless, it is clear that atorvastatin treatment shifts the balance of the cytokine milieu in the joints away from the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β) and towards the production of anti-inflammatory cytokines, such as IL-10.

Several studies have now demonstrated that chemokines, a group of cytokines with chemoattractant activity, are expressed in joints of arthritic patients (Koch et al., 1992; Villiger et al., 1992; Conti et al., 2002). Moreover, chemokines are also expressed in joints of experimental animals with arthritis and blockade of these mediators or of their receptors prevents or ameliorates arthritis progression (Gong et al., 1997; Bühl et al., 2004; Plater-Zyberk et al., 1997). For example, experiments in $CCL2^{-/-}$ mice or blockade of CCR2 (the receptor for CCL2) clearly demonstrates that CCL2 is relevant for arthritis progression (Gong et al., 1997; Bühl et al., 2004). There is also evidence for the role of CCL5 in experimental models of arthritis (Plater-Zyberk et al., 1997). Treatment with MetRANTES, a blocker of CCL5 receptors, reduced the severity of experimental arthritis in mice (Plater-Zyberk et al., 1997). In the present study, treatment with atorvastatin induced a significant inhibition of the local expression of CCL2 and CCL5 in joints of arthritic mice. This is consistent with the shown ability of atorvastatin to diminish chemokine concentration in tissue and in the circulation in other experimental models and in humans (Xu et al., 2003; Diomed et al., 2001). This is the first demonstration that statins prevent chemokine production in experimental arthritis. Thus, prevention of chemokine production adds to the list of inhibited mediators that could be contributing to the effects of atorvastatin treatment in experimental arthritis.

Joint pain is the most disabling symptom that affects the lives of patients with rheumatoid arthritis. Ideally and in addition to its anti-inflammatory effect, an agent used in the treatment of arthritis should ameliorate inflammatory pain. Here, we show that treatment with atorvastatin was associated with amelioration of inflammatory nociception in arthritic mice. It is likely that the ability of atorvastatin to ameliorate inflammatory nociception was secondary to its anti-inflammatory effects. Indeed, TNF-α is a central cytokine in mediating inflammatory pain (Sacks et al., 2001; Ferreira et al., 1998; Cunha et al., 1992) and atorvastatin partially prevented TNF-α release. However, the decreased nociception was already noticeable at a dose (1 mg/kg, data not shown) in which there was still some tissue inflammation (as assessed by joint swelling), suggesting that atorvastatin may affect inflammatory nociception independently. This latter finding clearly deserves further investigation.

There is much interest in the discovery of drugs that modify the progression of tissue destruction in rheumatoid arthritis. Here, we show that the treatment with atorvastatin after disease induction is efficient in modifying fundamental pathological processes in the joint, including neutrophil influx, tissue destruction and pro-inflammatory cytokine and chemokine production. The dose necessary for such effects (optimal at 10 mg/kg) is far greater than that necessary for the control of hypercholesterolemia in adults (around 40 mg/day). A recent study has shown an effect of atorvastatin treatment (40 mg daily over 6 month) on disease activity score in patients with rheumatoid arthritis (McCarey et al., 2004). In the latter study, there was also inhibition of some inflammatory markers (McCarey et al., 2004). In our experiments, there were noticeable effects at lower doses and statins have been developed and optimized for the treatment of hypercholesterolemia, not inflammation. Future studies must identify the exact mechanism(s) by which statins modify chronic inflammation as such mechanisms could clearly lead to novel therapeutic strategies for the treatment of arthritis.

Acknowledgements

This work was supported by Comissão de Aperfeiçoamento de Pessoal Nível Superior (CAPES/Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil) and Fundação de Amparo a Pesquisas do Estado de Minas Gerais (FAPEMIG/Brazil).

References

Arend, W.P., Dayer, J.M., 1995. Inhibition of the production and the effects of interleukin-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheum. 38, 151–160.

Bober, L.A., Rojas-Triana, A., Jackson, J.V., Leach, M.W., Manfra, D., Narula, S.K., Grace, M.J., 2000. Regulatory effects of interleukin-4 and interleukin-10 on neutrophil function ex vivo and on neutrophil influx in a rat model of arthritis. Arthritis Rheum. 43, 2660–2667.

Boe, A., Baiocani, M., Cabonatto, M., Papoian, R., Serlupi-Crescenzio, O., 1999. Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis. Cytokine 11, 1057–1064.

Bühl, H., Cihak, J., Schneider, M.A., Placchý, J., Rupp, T., Wenzell, I., Shakarami, M., Milz, S., Ellwart, J.W., Strangassinger, M., Schlöndorf, D., Mack, M., 2004. Dual role of CCR2 during initiation and progression of collagen-induced arthritis: evidence for regulatory activity of CCR2 T cell. J. Immunol. 172, 890–898.

Cannnetti, C.A., Leung, B.P., Culshaw, S., McInnes, I.B., Cunha, F.Q., Liew, F.Y., 2003. IL-18 enhanced collagen-induced arthritis by recruiting neutrophil via TNF-α and leukotriene B_4. J. Immunol. 171, 1009–1015.

Capetola, J.R., Shriver, A.D., Rosenthale, E.M., 1980. Suprofen, a new peripheral analgesic. J. Pharmacol. Exp. Ther. 214, 16–23.

Chen, H., Ikeda, U., Shimpo, M., Shimada, K., 2000. Direct effects of statins on cell primarily involved in athereosclerosis. Hypertens. Res. 23, 187–192.

Colpaert, F.C., Meert, T., De Witthand, P., Schmitt, P., 1982. Further evidence validating adjuvant arthritis as an experimental model of chronic pain in the rat. Life Sci. 31, 67–75.

Conti, P., Reale, M., Barbacane, R.C., Castellani, M.L., Orso, C., 2002. Differential production of RANTES and MCP-1 in synovial fluid from the inflamed human knee. Immunol. Lett. 80, 105–111.

Cunha, F.Q., Teixeira, M.M., Ferreira, S.H., 1992. The pivotal role of tumor necrosis factor α in the development of inflammatory hyperalgesia. Br. J. Pharmacol. 107, 660–664.

Diomed, L., Albani, D., Sottocorno, M., Donati, M.B., Bianchi, M., Fruscella, P., 2001. In vivo anti-inflammatory effect of statin is mediated by nonsterol mevalonate products. Arterioscler. Thromb. Vasc. Biol. 21, 1327–1332.

Feldmann, M., Brennan, F.M., Maini, R.N., 1996. Role of cytokine in rheumatoid arthritis. Annu. Rev. Immunol. 14, 397–440.

Feldmann, M., Brennan, F.M., Foxwell, B.M., Maini, R.N., 2001. The role of TNF alpha and IL-1 in rheumatoid arthritis. Curr. Dir. Autoimmun. 3, 188–199.

Ferreira, S.H., Lorenzetti, B.B., Bristow, A.F., Poole, S., 1998. Interleukin-1β as a potent hyperalgesic agent antagonized by a tripeptide analogue. Nature 334, 698–700.

Francischi, J.N., Pereira, L.M.S., Castro, M.S., 1997. Cyclosporin effects on hyperalgesia and oedema presented by arthritic rat: role of the central nervous system. Braz. J. Med. Biol. Res. 30, 101–111.

Francischi, J.N., Yokoro, C.M., Poole, S., Tafuri, W.L., Cunha, F.Q., Teixeira, M.M., 2000. Anti-inflammatory and analgesic effect of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis. Eur. J. Pharm. 399, 243–249.

Gong, J., Ratkay, L.G., Waterfield, J.D., Clark-Lewis, I., 1997. An antagonist of monocyte protein 1 (MCP-1) inhibits arthritis in the MLR-lrp mouse model. J. Exp. Med. 186, 131–137.

Ivashkiv, L.B., 1996. Cytokine expression and cell activation in inflammatory arthritis. Adv. Immunol. 63, 337–376.

Jonhson, B.A., Iacono, A.T., Zeevi, A., McCurry, K.R., Duncan, S.R., 2003. Statin is associated with improved function and survival of lung allografts. Am. J. Crit. Care Med. 167, 1271–1278.

Joosten, L.A., Helsen, M.M., Van De Loo, F.A., Van Den Berg, W.B., 1996. Anti-cytokine treatment of established type II collagen-induced arthritis in DBA/1 mice. A comparative study anti-TNF alpha, anti-IL-1 alpha/beta, and Il-1Ra. Arthritis Rheum. 39, 797–809.

Kanno, H., Hayashi, T., Esaki, T., Asai, Y., Thakur, N.K., Jayachandran, M., Iguchi, A., 1999. A HMG-CoA reductase inhibitor improved regression of atherosclerosis in the rabbit aorta without affecting serum lipid levels: possible relevance of up-regulation of endothelial NO synthesis mRNA. Biochem. Biophys. Res. Commun. 259, 414–419.

Kaplan, C., Valdez, J.C., Chandrasekaran, R., Eibel, H., Mikecz, K., Glant, T.T., Finnegan, A., 2002. Th1 and Th2 cytokine regulate proteoglycan-specific autoantibody isotypes and arthritis. Arthritis Res. 4, 54–58.

Katsikis, P.D., Chu, C.Q., Brennan, F.M., Maini, R.N., Feldmann, M., 1994. Immunoregulatory role of interleukin 10 in rheumatoid arthritis. J. Exp. Med. 179 (5), 1517–1527 (May 1).

Koch, A.E., Kunkel, S.L., Harlow, L.A., et al., 1992. Enhanced production of monocyte chemoattractant protein-1 in rheumatoid arthritis. J. Clin. Invest. 90, 772–779.

Kwak, B., Mulhaupt, F., Myit, S., Mach, F., 2000. Statin as a newly recognized type of immunomodulator. Nat. Med. 6, 13999–13402.

Kwak, B.R., Mulhaupt, F., March, F., 2003. Atherosclerosis: anti-inflammatory and immunomodulatory activities of statins. Autoimmum. Rev. 2, 332–338.

Lane, N.E., 1997. Pain management in osteoarthritis: the role of COX-2 inhibitors. J. Rheumatol. 24 (suppl. 49), 20–24.

Leung, B.P., Sattar, N., Crilly, A., Prach, M., McCarey, D.W., Payne, H., Madhok, R., Campbell, C., Gracie, J.A., Liew, F.Y., McInnes, I.B., 2003. A novel anti-inflammatory role for simvastatin in inflammatory arthritis. J. Immunol. 170, 1524–1530.

Liew, F.Y., McInnes, I.B., 2002. The role of innate mediators in inflammatory response. Mol. Immunol. 38, 887–890.

Maini, R.N., Taylor, P.C., 2000. Anti-cytokine therapy for rheumatoid arthritis. Annu. Rev. Med. 51, 207–229.

Matos, I.M., Souza, D.G., Seabra, D.G., Freire-Maia, L., Teixeira, M.M., 1999. Effects of tachykinin NK1 or PAF receptor blockade on the lung injury induced by scorpion venom in rats. Eur. J. Pharmacol. 376, 293–300.

McCarey, D.W., McInnes, I.B., Madhok, R., Hampson, R., Sherbakova, O., Ford, I., Capell, H.A., Sattar, N., 2004. Trial of atorvastatin in rheumatoid arthritis (TARA): double-blind, randomised placebo-controlled trial. Lancet 363, 2015–2121.

McKay, A., Leung, B.P., McInnes, I.B., Thompson, N.C., Liew, F.Y., 2004. A novel anti-inflammatory role of simvastatin in a murine model of allergic asthma. J. Immunol. 172, 2903–2908.

Moore, K.W., O'Garra, A., de Waal, M.R., Vieira, P., Mosmann, T.R., 1993. Interleukin-10. Annu. Rev. Immunol. 11, 165–190.

Moreland, L.W., Schiff, M.H., Baumgartner, S.W., Tindall, E.A., Fleischmann, R.M., Bulpitt, K.J., Weaver, A.L., Keystone, E.C., Furst, D.E., Meas, P.J., Ruderman, E.M., Horwitz, D.A., Arkfeld, D.G., Garrison, L., Burge, D.J., Blosh, C.M., Lange, M.L., MacDonnell, N.D., Weinblatt, M.E., 1999. Etanercept therapy in rheumatoid arthritis. A randomized, controlled trial. Ann. Intern. Med. 130, 478–486.

Nanki, T., Nagasaka, K., Hayashida, K., Saita, Y., Miyasaka, N., 2001. Chemokine regulated IL-6 and IL-8 production by fibroblast-like synoviocytes from patients with rheumatoid arthritis. J. Immunol. 167, 5381–5385.

Nishimoto, N., Yoshizaki, K., Maeda, K., Kuritani, T., Deguchi, H., Sato, B., Imai, N., Suemura, M., Kakehi, T., Takagi, N., Kishimoto, T., 2003. Toxicity, pharmacokinetics, and dose-finding study of repetitive treatment with the humanized anti-interleukin 6 receptor antibody MRA in rheumatoid arthritis. Phase I/II clinical study. J. Rheumatol. 30, 1426–1435.

Okouchi, M., Okayama, N., Omi, H., Imaeda, K., Shimizu, M., Fukutomi, T., Itoh, M., 2003. Cerivastatin ameliorates high insulin-enhanced neutrophil–endothelial cell adhesion and endothelial intercellular adhesion molecule-1 expression by mitogen-activated protein kinase activation. J. Diabetes its Complicat. 17, 380–386.

Pearson, C.M., 1956. Development of arthritis, periarthritis and periostitis in rats given adjuvant. Proc. Soc. Exp. Biol. Med. 91, 95–101.

Plater-Zyberk, C., Hoogewerf, A.J., Proudfoot, A.E.I., Power, C.A., Wells, T.N.C., 1997. Effect of a chemokine receptor antagonist on collagen induced arthritis in DBA/1 mice. Immunol. Lett. 57, 117–120.

Sacks, D., Cunha, F.Q., Poole, S., Ferreira, S.H., 2001. Tumor necrosis factor-α, interleukin-1β and interleukin-8 induced persistent mechanical nociception hypersensitivity. Pain 96, 89–97.

Schalwijk, J., Van der berg, W.B., Van de putte, L.B., Joosten, L.A., 1987. Elastase secreted by activated polymorphonuclear leukocytes causes chondrocyte damage and matrix degradation in intact reticular cartilage: escape from inactivation by α-1-proteinase inhibitor. Br. J. Phatol. 68, 81–88.

Swell, K.L., Trentham, D.E., 1993. Pathogenesis of rheumatoid arthritis. Lancet 341, 283–286.

Tatsuo, M.A.K.F., Carvalho, W.M., Silva, C.V., Miranda, A.E.G., Ferreira, S.H., Francischi, J.N., 1994. Analgesic and antiinflammatory effects of dipyrone in rat adjuvant arthritis model. Inflammation 18, 399–405.

Ugai, K., Ishikawa, H., Hirohata, K., Shirane, H., 1983. Interaction of polymorphonuclear with immune complex trapped in rheumatoid articular cartilage. Arthritis Rheum. 26, 1434–1441.

Van Roon, J.A., Lafeber, F.P., Bijlsma, J.W., 2001. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis. Arthritis Rheum. 44, 3–12.

Villiger, P.M., Terkeltaub, R., Lotz, M., 1992. Monocyte chemoattactant protein-1 (MCP-1) expression in human articular cartilage. J. Clin. Invest. 90, 488–496.

Xu, Z., Zhao, S., Li, Q., Zhou, H., 2003. Atorvastatin reduced plasma MCP-1 in patients with acute coronary syndrome. Clin. Chim. Acta 338, 17–24.

Zelvyte, I., Dominaitiene, R., Crisby, M., Janciauskiene, S., 2002. Modulation of inflammatory mediators and pparα and nfκb expression by pravastatin in response to lipoprotein in human monocyte in vitro. Pharmacol. Res. 45, 147–154.

Weitz-Schimidt, G., 2002. Statins as anti-inflammatory agents. Sciences 23, 482–487.

Youssef, S., Stüve, O., Patarroyo, J.C., Rulz, P.J., Radosevich, J.L., Hur, E.M., Bravo, M., Mitchell, D.J., Sobel, R.A., Steinman, L., Zamvill, S.S., 2002. The HMG-CoA reductase inhibitors, atorvastatin, promotes a Th2 bias and reverse paralysis in central nervous system autoimmune disease. Nature 420, 78–84.