Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 485 (2004) 1–10
www.elsevier.com/locate/ejphar

Cerivastatin inhibits proliferation of interleukin-1β-induced rat mesangial cells by enhanced formation of nitric oxide

Cornelia Blume[a,*], Danuta Sabuda-Widemann[a], Josef Pfeilschifter[b], Jörg Plum[a], K. Schrör[c], Bernd Grabensee[a], Karl-Friedrich Beck[b]

[a] *Klinik für Nephrologie und Rheumatologie Medizinische Einrichtungen, Heinrich-Heine-Universität, Moorenstrasse 5, 40225 Düsseldorf, Germany*
[b] *Pharmazentrum Frankfurt, Klinikum der Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany*
[c] *Institut für Klinische Pharmakologie und Pharmakologie, Heinrich-Heine-Universität, Düsseldorf, Germany*

Received 10 November 2003; accepted 13 November 2003

Abstract

The antiproliferative effect of statins on mesangial cells could represent a new therapeutic approach in glomerulonephritis. We studied in rat mesangial cells whether the antiproliferative action of cerivastatin on mesangial cells may be mediated by mesangial nitric oxide (NO) formation due to the inducible NO synthase (iNOS) or by induction of cyclooxygenase-2. Mesangial cells were stimulated with interleukin-1β and treated with cerivastatin for 24 h. Cell proliferation was examined by bromodeoxy-uridine (BrdU) incorporation, and nitrite and prostaglandin production was measured in supernatants as a means for iNOS or cyclooxygenase-2 activity. iNOS and cyclooxygenase-2 expression was quantified by Northern and Western blot analyses. Cerivastatin (0.0625 μM) significantly inhibited DNA synthesis in interleukin-1β-stimulated mesangial cells without altering cell viability. Interleukin-1β-induced nitrite production was twofold increased by 0.05 μM cerivastatin, and this effect could be reversed by addition of 100 μM mevalonate. iNOS mRNA levels increased sixfold (33% of maximum) in cerivastatin-treated mesangial cells as compared with vehicle-treated controls (3.5% of maximum). iNOS and cyclooxygenase-2 protein expression increased threefold (iNOS: 2.77 ± 0.53/cyclooxygenase-2: 3.49 ± 1.25). The NOS inhibitors *N*-methyl-L-arginine (L-NMMA) and L-*N*6-(1-iminoethyl)lysine (L-NIL) reversed the antiproliferative effect of cerivastatin. The cyclooxygenase-2 inhibitor celecoxib did not alter DNA synthesis and iNOS or cyclooxygenase-2 expression, but blocked prostacyclin production in interleukin-1β and cerivastatin-treated mesangial cells. In conclusion, cerivastatin increased cytokine-induced iNOS and cyclooxygenase-2 expression, thus constituting NO-regulated growth inhibition of mesangial cells.

Keywords: Mesangial cell, rat; Interleukin 1-β; Statin; Cerivastatin; NO (nitric oxide); NO (nitric oxide) synthase, inducible; Cyclooxygenase-2

1. Introduction

Autoregulation of renal mesangial cells alternates between growth stimulation and secretory activity and is paralleled by a balance between proliferative and growth inhibitory signals. Inflammation may lead to persisting mesangial cells activity during the breakdown of autoregulative, growth-controlling mechanisms and can induce progressive glomerular sclerosis. As a consequence, glomerular injury can become irreversible within the context of a glomerulonephritis (Haas et al., 1999). In vitro, it is well established that interleukin-1β displays such growth-promoting activities on mesangial cells and therefore can be used as an exemplary stimulus to mimic pathogenic processes of the inflamed kidney (Stahl et al., 1990). During rat anti-Thy1 nephritis, interleukin-1β has been recognised as a mesangial growth factor expressed by mesangial cells in vivo (Tesch et al., 1997) pointing to an autocrine inflammatory cascade as has been shown for glomerular epithelial cells (Tateyama et al., 2001). Interleukin-1β-activated mesangial cell proliferation may involve the induction of activator protein-2 (AP-2) as an important transcriptional factor (Suyama et al., 2001) via cytokine-induced formation of reactive oxygen species (Radeke et al., 1990; Huang and Domann, 1998).

By contrast, it was proposed, that cytokine-induced nitric oxide (NO) formation of mesangial cells (Pfeilschifter and Schwarzenbach, 1990) acts as an autocrine, antiproliferative regulator and inhibits the proliferation of cultured mesangial

* Corresponding author. Tel.: +49-211-8117726; fax: +49-211-8117722.
E-mail address: hblume@cityweb.de (C. Blume).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.11.043

cells (Garg and Hassid, 1993; Rupprecht et al., 2000) very likely by induction of apoptosis (Mühl et al., 1996). The NO-mediated apoptotic death of mesangial cells is antagonised by the simultaneous formation of superoxide (O_2^-) and vice versa (Brüne, 2002). The balanced and simultaneous generation of NO and O_2^- turned out to be protective for mesangial cells by regulation of cell turnover and growth in an inflammatory setting and therefore seems to be a possible target of drugs influencing mesangial cells proliferation.

NO production of mesangial cells is due to the activation of the inducible NO synthase (iNOS) since iNOS was immunohistochemically localized in the diseased kidney of patients with glomerulonephritis exclusively in mesangial cells, glomerular epithelial cells and infiltrating cells and very rarely in mesangial sections of control kidneys (Furusu et al., 1998). iNOS can be activated by interleukin-1β (Kunz et al., 1996) and amplified by endogenously produced nitric oxide (Mühl and Pfeilschifter, 1995). Once activated, NOS isoforms may not only produce NO but under certain conditions such as lack of the substrate L-arginine or the cofactor BH4 also superoxide or reactive nitrogen species, e.g., peroxynitrite ($ONOO^-$). Marnett (2000) reported a biochemical linkage of nitrite oxide biosynthesis with cyclooxygenase-2 activation mediated by peroxynitrite. Cyclooxygenase-2 induction was connected with a stimulation of endogenous prostaglandins presumably acting antiproliferative. In that way, NO serves as a regulator of cyclooxygenase-2 activity (Tetsuka et al., 1996). This regulation includes an "on-and-off" switching effect, depending on the NO level and on the stage of cellular activity (Diaz-Cazorla et al., 1999).

Since NO could have toxic or protective effects, there is as yet no clear understanding of how it affects the pathogenesis of glomerulonephritis. Cyclooxygenase-2 expression in this context is also controversially discussed. On the one hand, high-output NO and an increased cyclooxygenase-2 expression have been described as pathogenic factors in several glomerulonephritis models (Noris and Remuzzi, 1999; Blume et al., 1999). In contrast, the induction of iNOS during the chronic inflammatory phase of an experimentally induced glomerulonephritis in rats (anti-Thy1-nephritis) has been shown to be protective by interfering with intraglomerular coagulation processes (Westenfeld et al., 2002). Concerning cyclooxygenase-2, the use of selective cyclooxygenase-2 inhibitors in the same glomerulonephritis model led to a progression of the disease: renal damage was progressive and characterised by an increased albuminuria and mesangiolysis (Kitahara et al., 2002). Based on these observations, drugs capable of enhancing renal NO and subsequently cyclooxygenase-2 activity may be renoprotective in a variety of experimental renal diseases (Cattell, 2002).

Recent results concerning new therapeutic approaches for inflammatory renal diseases suggest that statins as inhibitors of the 3-hydroxy-3-methylglutaryl coenzyme A-reductase improve glomerular pathology and renal dysfunction, and this suppressive action on the progression of glomerular disease was observed in different models (Oda and Keane, 1999; Nogaki et al., 1999). In vitro observations of anti-inflammatory actions of statins on vascular cells displayed effects beyond lipid-lowering on the cellular level (Guijarro and Keane, 1996). Cell culture experiments have shown that statins suppress cell proliferation in many cell types, including mesangial cells (Nagasawa et al., 2000; Grandaliano et al., 1993), yet the underlying mechanisms are still unclear.

In the context of cholesterol-independent so-called pleiotropic effects of statins, it has been shown recently that the expression of cyclooxygenase-2 in vascular smooth muscle cells is mediated by statins via geranylgeranylated proteins (Degraeve et al., 2001). In addition, statins are involved in the expression of the endothelial NO synthase (eNOS) in vascular endothelial cells and therefore modulate NO production (Hernandéz-Perera et al., 1998). Similar results have been reported for vascular smooth muscle cells (Hattori et al., 2002).

Based on these data, we investigated whether cerivastatin influences cytokine-induced mesangial cell proliferation by affecting iNOS expression. Furthermore, the effect of cerivastatin on cyclooxygenase-2 protein expression was measured in interleukin-1β-stimulated mesangial cells. The detection of specific cellular effects of cerivastatin on the molecular level in vitro can possibly be transferred to the therapeutic action of this substance in vivo.

2. Methods

2.1. Chemicals

All chemicals not especially mentioned in the text were obtained from Sigma-Aldrich, Deisenhofen, Germany.

2.2. Rat mesangial cell culture and stimulation

Rat mesangial cells were cultivated as described previously (Pfeilschifter et al., 1993). Mesangial cells were grown in RPMI 1640 medium (Gibco, Mannheim, Germany, with addition of 100 U/ml penicillin, 100 μg/ml streptomycin and 5 μg/ml insulin) supplemented with 10% heat-inactivated fetal calf serum in six-well plates for RNA extraction, in 10-cm-diameter dishes for bromodeoxy-uridine (BrdU) assays and washed with phosphate-buffered saline (PBS) after 24 h. Afterwards, cells were maintained in serum-free Dulbecco's minimal essential medium (Life Technologies, Karlsruhe, Germany; DMEM with 0.45% glucose, 100 U/ml penicillin, 100 μg/ml streptomycin and 5 μg/ml insulin) supplemented with 0.1 mg/ml of fatty acid-free bovine serum albumin for 24 h before interleukin-1β stimulation (interleukin-1β, 0.5 nM, Novartis Pharma, Basel, Switzerland). Mesangial cells were used between passages 12 and 19. The following substances used in the experiments are water soluble, no vehicle had to be used: cerivastatin (kindly provided by Bayer, Wuppertal, Ger-

many; 0.0078–0.5 μM as indicated), *N*-methyl-L-arginine (L-NMMA) (0.5 mM), L-*N*6-(1-iminoethyl)lysine (L-NIL) (0.5 mM), L-/D-arginine (10 mM). Celecoxib (kindly provided by Pfizer (New York, USA, 5–10 μM as indicated) and 2-(4-morpholino)-8-phenyl-4*H*-1-benzopyran-4-one (LY 294002, Calbiochem-Novabiochem, Schwalbach, Germany; 0.1–10 μM as indicated) had to be solved in dimethylsulfoxide (DMSO); here the corresponding controls were pretreated with the vehicle.

2.3. Preparation of mevalonate for use in cell culture

The inactive form of mevalonate was converted to its active form as described (O'Donnell et al., 1993). In brief, 130 mg of the inactive mevalonate was dissolved in 3.25 ml absolute ethanol, and 0.1 M NaOH was added, heated at 50 °C for 2 h and neutralized with 0.1 M HCl to pH 7.4.

2.4. Cell viability and cytotoxicity of mesangial cells

Cell viability and cytotoxicity of mesangial cells were determined under the chosen experimental conditions by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide test (MTT, Boehringer-Mannheim, Mannheim, Germany) (Mosmann, 1983) and lactate dehydrogenase (LDH) activity in the mesangial cell culture medium. According to the criteria of the MTT test, cell viability was measured and compared to unstimulated controls (= 100%, mesangial cells in RPMI medium with 1% fetal calf serum) and was above 80% under all experimental conditions studied except addition of cerivastatin concentrations ≥ 0.1 μM. Therefore, the potential toxicity of this substance was distinctively evaluated using a cytotoxicity detection kit (Roche Molecular Biochemics, Mannheim, Germany). Mesangial cells incubated with increasing concentrations of cerivastatin for 24 h were tested strictly in accordance with the instructions for the kit. Cell damage indicating LDH activity is determined in an enzymatic test and is proportional to the reduction of NAD^+ to $NADH^+/H^+$ and to the transfer of H^+ to the tetrazolium salt 2-(4-iodophenyl)-5-(4-nitrophenyl)-3-phenyltetrazolium (INT), which is reduced to formazan in this assay. Formazan was detected photometrically at 490 nm.

2.5. Analysis of mesangial cell proliferation

Cell proliferation was quantified by measuring DNA synthesis using the cell incorporation of bromodeoxy-uridine (Boehringer-Mannheim) (Selden et al., 1993).

2.6. Preparation of mesangial cells for total RNA extraction

Differentially treated mesangial cells were harvested for total RNA extraction strictly in accordance with the instructions for the RNeasy-Mini-Kit (Qiagen, Hilden, Germany). RNA was quantified by spectrometry, and RNA integrity was monitored by agarose gel electrophoresis.

2.7. Nitrite analysis

The activity of iNOS was measured by determination of nitrite, one of the stable end products of NO formation, in the culture medium of mesangial cells using the Griess method (Green et al., 1982) with a ready-to-use reagent (Merck, Darmstadt, Germany). Nitrite levels were expressed in picomoles NO_2^- per microgram of total protein determined by Bradford analysis.

2.8. Northern blot analysis of iNOS mRNA

Isolation of total RNA from mesangial cells and Northern blotting was performed as described previously (Chomczynski and Sacchi, 1987). Filter-bound RNA was hybridised using a [^{32}P]-labelled cDNA probe for iNOS. Equivalent loading of the RNA samples was corrected after rehybridization of the filter with a cDNA probe for GAPDH. The mRNA levels for iNOS and GAPDH were measured by a Fujifilm BAS 1500 automated detector system (Raytest, Straubenhardt, Germany). iNOS mRNA levels were corrected for the mRNA levels of GAPDH and are expressed as arbitrary units.

2.9. Western blot analysis of cyclooxygenase-2 and iNOS protein expression

Confluent mesangial cells grown in 10-cm-diameter dishes were stimulated with 0.5 nM interleukin-1β for 24 h after preincubation with cerivastatin (0.005–0.1 μM) for 30 min. Thereafter, mesangial cells were homogenised using homogenisation buffer (20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 1mM EGTA, 1% Triton X-100, 25 μg leupeptin, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiotreitol). After incubation on ice for 5 min, cell extracts were sonicated three times for 10 s and centrifuged at 13,000 rpm for 2 min. The protein concentration of the cell lysate was determined using the Bradford protein assay (Bio-Rad, Munich, Germany). Samples containing a total of 10 μg protein were incubated at 95 °C for 5 min and subjected to a sodium dodecyl sulfate (SDS)-polyacrylamide gel, 8% (wt/vol) for iNOS, 12% (wt/vol) for cyclooxygenase-2. After electrophoresis, proteins were blotted onto nylon membranes, and the immunoreactive protein was detected using specific antibodies against the iNOS [N-terminal antibody (Kunz et al., 1994) in a dilution of 1:2500], cyclooxygenase-2 (monoclonal antibody, 1:500, Transduction Laboratories). Protein bands were visualised using horseradish peroxidase-linked secondary antibodies and increased chemiluminiscence (Amersham Pharmacia Biotech Europe, Freiburg, Germany).

The intensity of the bands representing iNOS (130 kDa) and cyclooxygenase-2 (70 kDa) was evaluated using Quantity One (Biometra, Munich, Germany) for densitometrical analysis, whereby the ratio with α-actin was used to correct for equivalent loading (detected using specific antibodies against the 26-kDa protein of α-actin).

2.10. Western blot analyses of phospho-PKB and PKBα/Akt

Quiescent mesangial cells were treated with cerivastatin (0.1 μM) for short time intervals up to 16 min. Thereafter, cells were harvested and quickly dissolved in 2 × concentrated electrophoresis sample buffer (125 mM Tris pH 6.8, 4% SDS, 10% glycerol, 0.006% bromophenol blue, 2% β-mercaptoethanol) for protein analysis. Western blots were performed using specific phospho-Ser473-PKB antibody (New England Biolabs, Frankfurt am Main, Germany) and total PKBα antibody (Upstate Biotechnologies, Lake Placid, USA) at a dilution of 1:1000 and 1: 1600, respectively. Bands (60 kDa) were detected by the ECL method according to the manufacturer's recommendation (Amersham Pharmacia Biotech Europe).

2.11. Prostaglandin measurement in cell culture supernatants

6-Keto-$PGF_{1\alpha}$ and thromboxane B_2 as stable metabolites of the COX-2-dependent prostaglandins prostacyclin and thromboxane A_2 and PGE_2 were determined using radioimmune-assays (RIAs) as previously described (Schrör and Seidel, 1988). For this purpose, MC were stimulated with interleukin-1β (0.5 nM) with or without cerivastatin (0.05–0.1 μM) for 24 h, and cell supernatants were frozen immediately at −20 °C. Corresponding samples were additionally treated with the COX-2-specific inhibitor celecoxib (5 μM). Three independent experiments showed similar results.

3. Results

3.1. Cytotoxicity of cerivastatin

To test drug toxicity on mesangial cells, lactate dehydrogenase (LDH) activity as a marker for cell lysis was measured in the cell-free supernatant of mesangial cells after drug incubation over 24 h. Cerivastatin concentrations of 0.25 μM and above appeared to be toxic, leading to LDH release of 35% of the maximum of Triton X 100-induced cell lysis (corresponding to an optical density of 0.572 compared to a maximal OD of 1.64). In contrast, LDH release remained at 12.8% of the maximum at concentrations up to 0.125 μM (corresponding to an OD of 0.211, not shown), compared to the vehicle-treated controls (OD 0.125). Parallel incubation of mesangial cells with interleukin-1β and cerivastatin resulted in a LDH release of 20% of the maximum (corresponding to an OD of 0.344). Higher cerivastatin concentrations than 0.125 μM caused a twofold increase of cytotoxicity in interleukin-1β-treated mesangial cells. We concluded from these data that cerivastatin concentrations up to 0.1 μM were "nontoxic" for interleukin-1β-stimulated mesangial cells and were used for all further experiments.

3.2. Antiproliferative effect of cerivastatin on DNA synthesis of interleukin-1β-stimulated mesangial cells

As a marker for cell proliferation, DNA synthesis was measured by incorporation of bromodeoxy-uridine into mesangial cells. Mesangial cells stimulated with 1 nM interleukin-1β for 24 h showed a 3.5-fold increase in DNA synthesis (optical density 1.4) and a twofold increase in DNA synthesis at 0.5 nM interleukin-1β (OD of 0.8) compared with unstimulated controls (OD 0.4). This equals to approximately 40% of DNA synthesis in mesangial cells stimulated with 10% fetal calf serum (OD 1.9) and 50% of DNA synthesis in mesangial cells stimulated with platelet-derived growth factor (PDGF) (Fig. 1), and 0.5 nM interleukin-1β was used in all further experiments.

Cerivastatin showed an antiproliferative effect on mesangial cells stimulated with 10% fetal calf serum (Fig. 2) with an IC_{50} of 0.35 μM and significantly reduced interleukin-1 β-driven DNA synthesis of mesangial cells ($P < 0.01$) at concentrations of 0.0625 μM and above compared with vehicle-treated controls (Fig. 2). The basal proliferation of unstimulated mesangial cells was not affected by cerivastatin.

3.3. Addition of the NOS inhibitors L-NMMA and L-NIL led to a significant reversal of the antiproliferative cerivastatin effect

Addition of the NOS inhibitors L-NMMA (0.5 mM, Fig. 3) or L-NIL (0.5 mM, Fig. 3) significantly reversed the antiproliferative effect of nontoxic cerivastatin concentrations on interleukin-1β-induced DNA synthesis. In contrast, proliferation of interleukin-1β-stimulated mesangial cells was not affected by addition of NOS-inhibitors in the absence of cerivastatin (Fig. 3). Addition of an excess of L-arginine (10 mM) to interleukin-1β and cerivastatin-treated mesangial cells significantly antagonised the effect of L-NMMA, whereas addition of the inactive D-arginine (10 mM) did not.

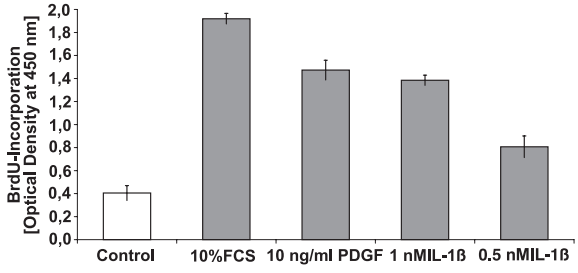

Fig. 1. BrdU incorporation in rat mesangial cells stimulated with different agonists. Growth-arrested mesangial cells were stimulated for 24 h with vehicle (open bars, control), 10% fetal calf serum (FCS), 10 ng/ml PDGF or 1 nM/0.5 nM interleukin-1β (IL-1β) as indicated. Bromodeoxy-uridine (BrdU) incorporation was measured over a time period of 2 h ($n = 6$, means ± S.D.). In mesangial cells stimulated with 10% fetal calf serum, the IC_{50} of cerivastatin was determined at 350–400 nM.

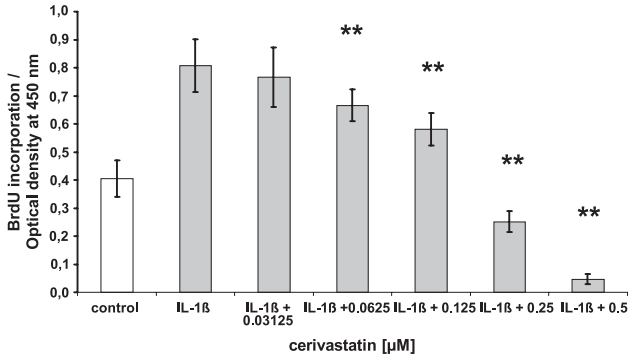

Fig. 2. Effect of cerivastatin on BrdU incorporation in interleukin-1β-stimulated rat mesangial cells. Bromodeoxy-uridine (BrdU) incorporation was measured over a time period of 2 h in interleukin-1β (IL-1β)-stimulated (filled bars) and -unstimulated mesangial cells (control, open bars). Cerivastatin concentrations were as indicated. Statistical analysis was performed using simple ANOVA and Dunnett's test ($n = 6$, means ± S.D.). $^{**}P < 0.01$ versus interleukin-1β.

3.4. Effect of cerivastatin on interleukin-1β-induced iNOS activity and iNOS expression

The activity of iNOS in mesangial cells was measured by determination of nitrite, one of the stable end products of NO formation, using the Griess method, and expressed in picomoles nitrite per microgram of total protein. Mesangial cells stimulated with interleukin-1β were coincubated with cerivastatin and showed increased iNOS activity. Nitrite production of cerivastatin and interleukin-1β-treated mesangial cells was significantly higher than in interleukin-1β-stimulated mesangial cells in the absence of cerivastatin. Nitrite levels reached a maximum of 91 pmol NO_2/µg protein at 0.1 µM cerivastatin and 79 pmol NO_2/µg protein at 0.05 µM cerivastatin as compared with 30 pmol NO_2/µg protein in interleukin-1β-stimulated controls (Fig. 4A). Cerivastatin alone did not cause significant nitrite production (data not shown). Addition of the NOS inhibitor L-NMMA (0.5 mM) abolished cerivastatin-driven nitrite production after interleukin-1β to 7 pmol/µg protein. The addition of an excess of L-arginine (10 mM) partially reversed the inhibitory effect of L-NMMA nitrite producing 19 pmol/µg protein, whereas addition of D-arginine (10 mM) was without effect. Addition of 100 µM mevalonate

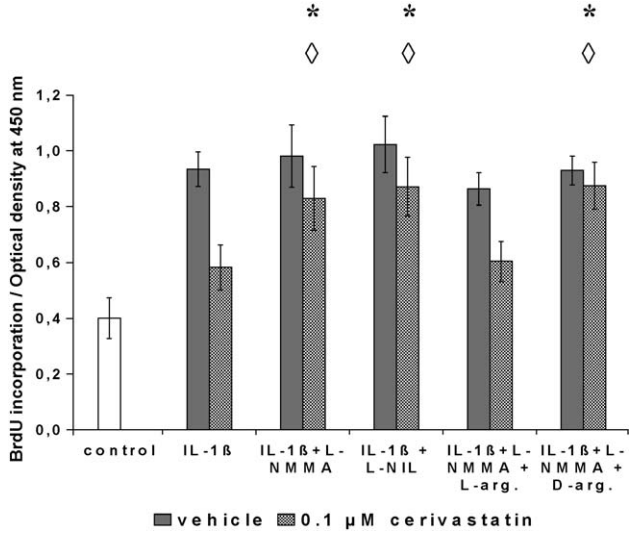

Fig. 3. Effect of L-NIL and L-NMMA and L-/D-arginine on BrdU incorporation in interleukin-1β- and cerivastatin-treated rat mesangial cells. Bromodeoxy-uridine (BrdU) incorporation was measured over a time period of 2 h in unstimulated mesangial cells (control, open bars), in interleukin-1β-stimulated mesangial cells without cerivastatin (filled bars) or in interleukin-1β-stimulated mesangial cells with 0.1 µM cerivastatin (hatched bars). L-NMMA (0.5 mM), L-NIL (0.5 mM) or L-/D-arginine (10 mM) were added as indicated. Statistical analysis was performed using simple ANOVA and Dunnett's test ($n = 6$, means ± S.D.). $^{*}P < 0.05$ versus interleukin-1β + cerivastatin. ◊ $P < 0.05$ versus L-arginine.

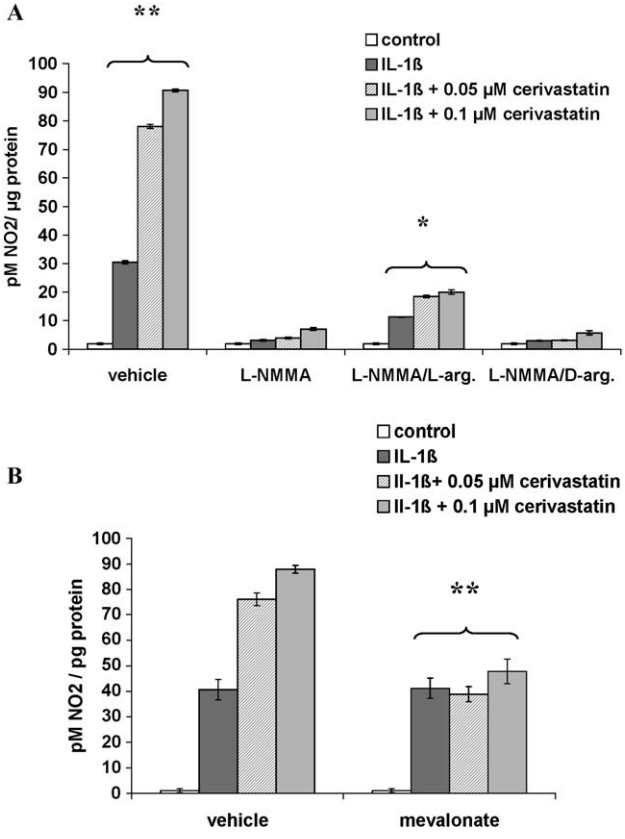

Fig. 4. (A) Effect of L-NMMA and L-/D-arginine on iNOS activity in interleukin-1β- and cerivastatin-treated rat mesangial cells. iNOS activity of unstimulated (control, open bars) or interleukin-1β-treated mesangial cells (dark grey bars) or interleukin-1β- and cerivastatin-treated mesangial cells (hatched and grey bars) was measured by determination of mesangial nitrite production, one of the stable end products of NO formation [nitrite (pmol/µg protein), $n = 6$; means ± S.D.]. L-NMMA (0.5 mM), L-arginine (10 mM), D-arginine (10 mM) were added as indicated. Statistical analysis was performed using ANOVA and Dunnett's test. $^{*}P < 0.05$ or $^{**}P < 0.01$ versus interleukin-1β + L-NMMA. (B) Effect of mevalonate on iNOS activity in interleukin-1β- and cerivastatin-treated rat mesangial cells. iNOS activity of unstimulated (control, open bars) or interleukin-1β-treated mesangial cells (IL-1β, squared bars) or interleukin-1β- and cerivastatin-treated mesangial cells (hatched and grey bars) was measured as described [nitrite (pmol/µg protein), $n = 6$; means ± S.D.]. Mevalonate (100 µM) was added as indicated. Statistical analysis was performed using ANOVA and Dunnett's test. $^{**}P < 0.01$ versus interleukin-1β ± cerivastatin.

led to abolishment of the cerivastatin-induced mesangial nitrite production in interleukin-1β-treated MC (Fig. 4B).

iNOS mRNA steady-state levels were investigated by Northern blot analysis. iNOS mRNA was detectable in interleukin-1β-stimulated mesangial cells, whereas no iNOS mRNA expression was observed in unstimulated control cells. Cerivastatin incubation alone did not cause iNOS mRNA induction in mesangial cells (Fig. 5A, lanes 1 and 2). Cerivastatin dose-dependently amplified interleukin-1β-induced iNOS mRNA expression in a range from 0.005 up to 0.1 μM cerivastatin (Fig. 5A, lanes 3–6). Basal induction of iNOS mRNA at 0.5 nM interleukin-1β is minimal (3.5% of maximum), but this experimental condition was suited to show the increasing effect of cerivastatin treatment. Using GAPDH as an internal standard, we densitometrically analysed iNOS mRNA expression in cytokine-treated mesangial cells and found that iNOS mRNA was significantly increased by sixfold after addition of cerivastatin (0.05 μM) as compared to vehicle-treated controls. Concomitantly,

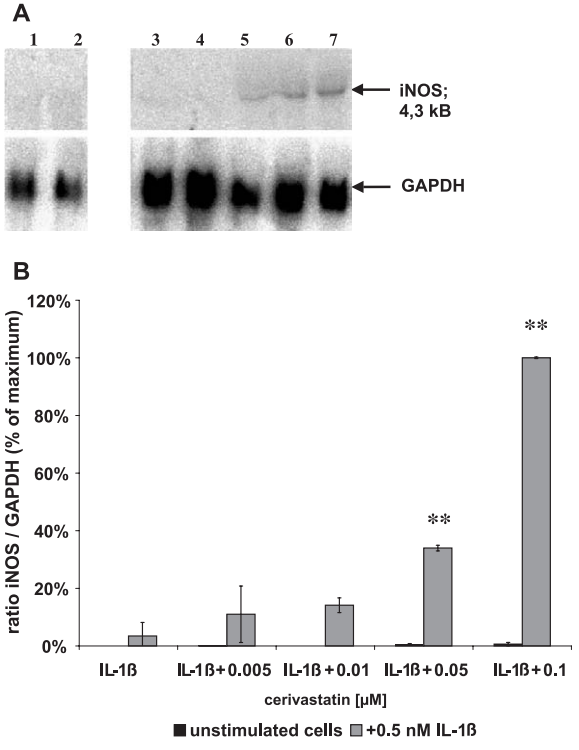

Fig. 5. iNOS mRNA steady-state levels in mesangial cells treated with interleukin-1β and cerivastatin. iNOS (4.3 kb) and GAPDH mRNA levels were measured using Northern blot analysis (A), 30 μg mRNA was used per lane as described in Methods. Lane 1: unstimulated mesangial cells (control); lane 2: mesangial cells incubated with cerivastatin (0.1 μM). Lanes 3–7 represent interleukin-1β-stimulated mesangial cells with increasing concentrations of cerivastatin. Lane 3: control lane; lane 4: 0.005 μM; lane 5: 0.01 μM: lane 6, 0.05 μM: lane 7, 0.1 μM. To correct for differences in loading, iNOS mRNA levels in interleukin-1β-stimulated and/or drug-treated cells were corrected for the mRNA levels of GAPDH and are expressed as percent of the maximum value [(B), $n=3$, means ± S.D.]. Statistical analysis was performed using ANOVA. $^{**}P<0.01$ versus interleukin-1β.

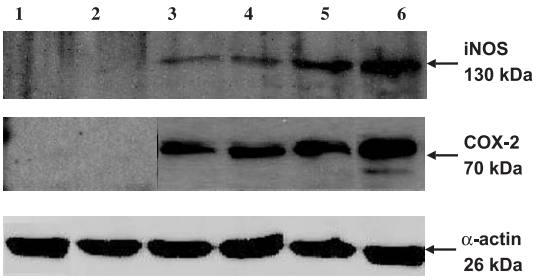

Fig. 6. iNOS and cyclooxygenase-2 protein expression in interleukin-1β- and cerivastatin-treated mesangial cells. All lanes contain a total of 10 μg protein from mesangial cells solubilised according to the experimental protocol described earlier. Western blot was performed using specific antisera. Lanes 1 and 2 contain unstimulated mesangial cells with (lane 2) or without (lane 1) 0.1 μM cerivastatin. Lanes 3–6 represent interleukin-1β-stimulated mesangial cells treated with increasing concentrations of cerivastatin (lane 3: control lane; lane 4: 0.005 μM cerivastatin; lane 5: 0.05 μM cerivastatin; lane 6: 0.1 μM cerivastatin).

iNOS mRNA was increased by 20-fold in interleukin-1β-treated mesangial cells at 0.1 μM cerivastatin ($^{**}P<0.01$; Fig. 5B).

Western blot analysis showed that in cerivastatin-treated mesangial cells, interleukin-1β-induced iNOS protein expression was significantly and dose-dependently increased (Fig. 6). Exemplary, in interleukin-1β-stimulated mesangial cells treated with 0.05 μM cerivastatin, the increase was threefold as compared to interleukin-1β-stimulated mesangial cells without cerivastatin ($^{*}P<0.05$; Fig. 8).

Addition of the NOS-inhibitor L-NMMA (0.5 and 1 mM) led to a significant decrease of iNOS protein expression in interleukin-1β-treated mesangial cells at 0.05 μM cerivastatin as compared to the corresponding protein samples without L-NMMA (Figs. 7 and 8). In summary, cerivastatin led to a statistically significant increase in iNOS activity, iNOS mRNA expression and iNOS protein expression after interleukin-1β stimulation of mesangial cells. This increase was reversible by L-NMMA. Addition of the selective cyclooxygenase-2 inhibitor celecoxib (up to 10 μM) did not alter iNOS expression or activity in these experimental settings (data not shown).

3.5. Effect of cerivastatin on interleukin-1β-induced cyclooxygenase-2 protein expression

Cyclooxygenase-2 protein expression in interleukin-1β-stimulated mesangial cells stimulated showed a threefold, dose-dependent increase after incubation with 0.05 μM cerivastatin (Figs. 6 and 8). This increase in cyclooxygenase-2 protein expression was statistically significant ($^{**}P<0.01$) as compared with untreated controls, interleukin-1β-stimulated mesangial cells or mesangial cells treated with cerivastatin alone (Fig. 7).

The addition of the NOS inhibitor L-NMMA (0.5–1 mM) led to a significant decrease of cyclooxygenase-2 expression after interleukin-1β stimulation, but not after additional cerivastatin treatment (Figs. 6 and 7). Addition of

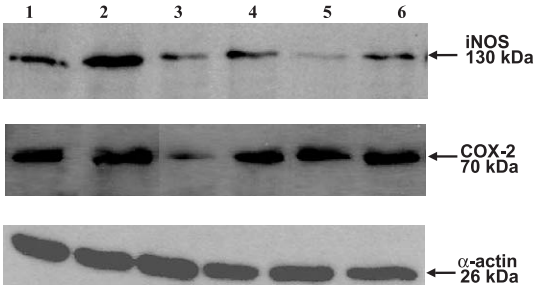

Fig. 7. Effect of L-NMMA on iNOS and cyclooxygenase-2 protein expression in interleukin-1β- and cerivastatin-treated mesangial cells. All lanes contain a total of 10 μg protein from interleukin-1β-stimulated mesangial cells solubilised as described in Methods. Western blot was performed using specific antisera. Lane 1: control; lane 2: 0.05 μM cerivastatin. Lane 3: 0.5 mM L-NMMA; lane 4: 0.05 μM cerivastatin and 0.5 mM L-NMMA. Lane 5: 1 mM L-NMMA; lane 6: 0.05 μM cerivastatin and 1 mM L-NMMA.

up to 10 μM celecoxib alone had no effect on cyclooxygenase-2 expression in this experimental setting (data not shown).

3.6. Cylooxygenase-2 upregulation is protective but not growth regulating

The cerivastatin effect includes upregulation of cyclooxygenase-2 protein in interleukin-1β-stimulated mesangial cells. However, BrdU incorporation of interleukin-1β-stimulated and cerivastatin-treated mesangial cells was not significantly altered by the addition of celecoxib (up to 10 μM, results not shown).

Cytotoxicity of interleukin-1β-treated mesangial cells according to LDH release induced by cerivastatin was not influenced by addition of the NOS-inhibitor L-NIL (2 mM, results not shown). Addition of the cyclooxygenase-2 specific inhibitor celecoxib (5 μM) in the above mentioned experimental setting led to a significantly increased cytotoxicity (*$P<0.05$) at 0.1 μM cerivastatin as compared to interleukin-1β- and vehicle-treated mesangial cells (Fig. 9). Celecoxib alone did not cause significant cytotoxicity in mesangial cells (data not shown). Cerivastatin led to a dose-dependent and significant enhancement of the COX-2-dependent prostacyclin formation up to sixfold at 0.1 μM cerivastatin in interleukin-1β-treated MC (Fig. 10). In contrast, thromboxane A_2 and PGE_2 were not detectable under these experimental conditions. Whereas COX-2 protein expression remained unchanged, mesangial prostacyclin production was completely abolished by addition of celecoxib.

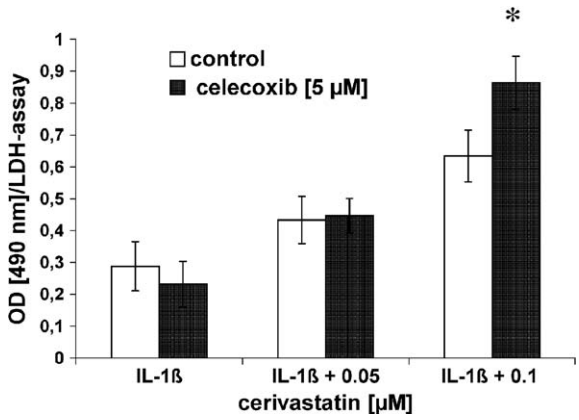

Fig. 9. LDH activity in the supernatants of interleukin-1β- and drug-treated mesangial cells. LDH activity was determined at 490 nm in the cell-free culture supernatant of interleukin-1β and cerivastatin (open bars) or in IL-1β- and celecoxib-treated mesangial cells as indicated (closed bars). Statistical analysis was performed using ANOVA ($n=6$; means ± S.D.). **$P<0.05$ versus interleukin-1β + cerivastatin.

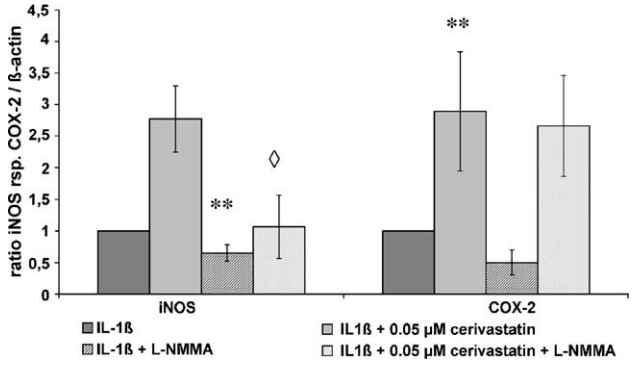

Fig. 8. Densitometrical analysis of iNOS and cyclooxygenase-2 protein expression after interleukin-1β stimulation, with or without cerivastatin, with or without L-NMMA. The protein expression of iNOS and cyclooxygenase-2 in mesangial cells stimulated with 0.5 nM interleukin-1β (IL-1β) was set to 1. Cerivastatin and L-NMMA were added as indicated. Protein bands obtained by Western blotting were analysed by densitometry and are expressed as arbitrary units compared with controls (ANOVA, $n=3$; means ± S.D.); **$P<0.01$ versus interleukin-1β; ◊$P<0.01$ versus interleukin-1β + cerivastatin.

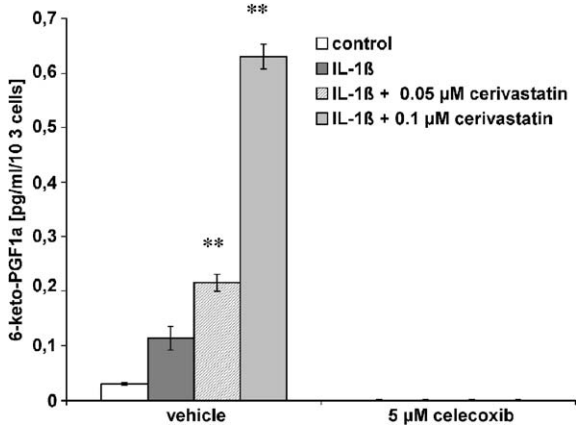

Fig. 10. COX-2 dependent prostaglandins in the supernatant of interleukin-1β- and drug-treated mesangial cells. 6-Keto-$PGF_{1\alpha}$ was measured as a stable metabolite of prostacyclin using RIA in cell culture supernatants. To this end, therefore, MC were stimulated for 24 h with vehicle (control), interleukin 1β and cerivastatin as indicated in the absence and presence of celecoxib (5 μM). Results are of three independent experiments, means ± S.D.; **$P<0.01$ versus interleukin 1β, ANOVA.

4. Discussion

Recently, statins have demonstrated to exert beneficial effects in different models of progressive renal failure. Some of the effects of statins have been reported to be independent of cholesterol reduction. Simvastatin suppressed cell proliferation in rats with anti-Thy 1.1-nephritis (Yoshimura et al., 1999) and PDGF-induced mesangial cells proliferation in vitro (Grandaliano et al., 1993). This antiproliferative effect was not connected to the alteration in PDGF mRNA steady-state levels. In line with these results, cerivastatin significantly inhibited serum-derived mesangial cells proliferation (Fig. 1). Interleukin-1β-induced mesangial cell proliferation was reduced under cerivastatin (Fig. 2) without apparent cytotoxicity up to 0.1 μM. This observation is crucial since cerivastatin was withdrawn from the market throughout the world 2 years ago due to unspecific muscle damaging and rhabdomyolysis, and this side effects seemed to be dose dependent.

It was the aim of this study to examine the underlying mechanism of the antiproliferative effect of cerivastatin on cytokine-stimulated mesangial cells as a model for the inflamed kidney. Interleukin-1β is considered to be a main stimulus for glomerular mesangial cells to produce nitric oxide via the inducible NO synthase (Pfeilschifter and Schwarzenbach, 1990). Several studies showed that high output NO inhibited the proliferation of mesangial cells (Rupprecht et al., 2000; Hruby and Beck, 1997). Our results suggest that the antiproliferative effect of cerivastatin on interleukin-1β-stimulated mesangial cells is significantly mediated by cerivastatin-induced NO production by mesangial cells challenged with cytokines. We could show that NO production in interleukin-1β- and cerivastatin (up to 0.1 μM)-treated mesangial cells was not associated with cell lysis, but with significantly reduced DNA synthesis (Figs. 2 and 3). Nontoxic amounts of cerivastatin dose-dependently increased nitrite synthesis two- to threefold (Fig. 4 and iNOS mRNA levels 6- to 18-fold (Fig. 5) and protein levels iNOS mRNA levels threefold (Figs. 6 and 8) as compared to interleukin-1β-stimulated controls. The increase in iNOS activity caused by cerivastatin after interleukin-1β induction of mesangial cells was reversed by the addition of L-NMMA, and this reversal in turn was abolished by L-arginine. In parallel to that, the observed increase of iNOS protein expression by cerivastatin was reversed by NO inhibition, indicating that cerivastatin interferes with NO-induced iNOS protein expression in cytokine-stimulated cells (Mühl and Pfeilschifter, 1995). In contrast to our results, Park et al. (2000) suggested that the therapeutic effect of cerivastatin in rats transgenic for angiotensinogen might be associated with suppressed iNOS expression and considered this aspect as beneficial. Since angiotensin II has been shown to inhibit iNOS expression, these results may be due to the different experimental settings (Kihara et al., 1999).

Modulation of NO by statins has been shown earlier. Atorvastatin and simvastatin prevented the reduction in mRNA expression and protein levels of endothelial nitric oxide synthase after exposure to oxidised low-density lipoproteins (LDL) (Hernandéz-Perera et al., 1998). In contrast, cerivastatin treatment of unstimulated mesangial cells alone did not lead to a significant nitrite formation. The observed cerivastatin effect on mesangial iNOS expression and activity in our experiments was depending upon preactivation of the enzyme, e.g., by treatment of mesangial cells with interleukin-1β (Mühl and Pfeilschifter, 1995). In parallel, we observed that fluvastatin enhanced activity of iNOS in terms of nitrite production in interleukin-1β-treated mesangial cells (results not shown), suggesting that the effects observed are specific for the group of statins.

The mechanism by which cerivastatin affects iNOS and cyclooxygenase-2 expression may involve inhibition of geranylgeranylation via the mevalonate pathway (Degraeve et al., 2001), and consequently addition of sufficient mevalonate led to suppression of cerivastatin-induced nitrite production in cytokine-activated MC (Fig. 4B). These results point to a possible role of Rho-GTPases that get active as geranylgeranylated proteins and regulate gene expression. Recent results have shown that in the presence of an effective dose of a statin, Rho-GTPases are inactivated (Stamatakis et al., 2002). Furthermore, a lowered iNOS and cyclooxygenase-2 expression was observed under the influence of Rho inactivators (Degraeve et al., 2001; Hausding et al., 2000). In mesangial cells, simvastatin acts antiproliferative by inactivation of Rho-GTPases and the cyclin-dependent kinase p21 under high-glucose conditions, implicating a therapeutic role in early stages of diabetic nephropathy (Danesh et al., 2002). Another possible pharmacological pathway targeted by statins may be the activation of nuclear factor κB (NFκB). However, Hattori et al. (2002) showed that although statins slightly modulate interleukin-1β-induced NFκB activation, this effect was not associated with their effect on NO production. To address a possible role for the PI-3/PKB pathway, we stimulated MC with different concentrations of cerivastatin for up to 16 min. No changes in PKB phosphorylation could be detected between controls and cerivastatin-treated cells. To exclude a downregulation of PKB phosphorylation by cerivastatin, we prestimulated resting MC with 0.5 μM cerivastatin for 30 min and forced mesangial cells in the presence of 1% FCS to PKB phosphorylation. No inhibitory effect of cerivastatin prestimulation on FCS-dependent PKB phosphorylation was detectable. In long-time experiment (24 h), the PI-3 kinase inhibitor LY 294002 showed no effect on cytokine- and cerivastatin-induced iNOS expression (results not shown). These data suggest that PKB most likely is not involved in cerivastatin-evoked signalling in mesangial cells.

Fig. 7 shows, that the increase of cyclooxygenase-2 protein expression induced by interleukin-1β was NO dependent and could be reversed by addition of the NOS inhibitors. Interestingly, the additional enhancement of cycloxygenase-2 protein expression evoked by cerivastatin could not be inhibited by the NOS inhibitors. This

observation suggests a different mode of action for cyclooxygenase-2 induction by cerivastatin as compared to interleukin-1β.

Cylooxygenase-2 protein expression was also not affected by celecoxib, although the production of prostacyclin was completely inhibited (Fig. 10). It was shown earlier that the inhibitory effect of NSAIDS on cyclooxygenase-dependent prostanoid synthesis is not connected to a suppressed cyclooxygenase expression but due to an inhibition of enzymatic activity (Klein et al., 1994). The functional role of cyclooxygenase-2 is still controversially discussed. In cytokine-stimulated endothelial cells, the high-density lipoproteins (HDL) induced increase of cyclooxygenase-2 protein and subsequently prostacyclin formation was considered beneficial and associated with antiatherogenic effects (Cockerill et al., 1995). In line with these results, we observed an upregulation of prostacyclin synthesis in supernatants of cytokine-stimulated mesangial cells after cerivastatin treatment.

Cytotoxicity measured by determination of LDH release was significantly increased in interleukin-1β- and cerivastatin-treated mesangial cells under coincubation with celecoxib (Fig. 9), thus pointing to a protective role of the statin-induced cyclooxygenase-2 upregulation. The cytoprotective effect of cyclooxygenase-2 may include prevention of NO-induced cell necrosis (Ishaque et al., 2003), mediated by enhanced production of prostacyclin.

We suggest that the action of cerivastatin on cell growth is NO mediated. Cerivastatin enhanced the cytokine-induced endogenous NO formation via iNOS and thereby led to a decreased mesangial cells proliferation. Moreover, NO-triggered apoptosis may further promote this situation. Additional studies will have to clarify this point. The cerivastatin-induced iNOS and cyclooxygenase-2 expression in mesangial cells may help to optimise renal hemodynamics and to counterbalance cytokine-induced proliferation and oxidative stress of mesangial cells in the inflamed kidney and may be beneficial in therapy of renal disease.

Acknowledgements

We thank PD Dr. A. Huwiler for helpful discussion and advise concerning PKB/Akt detection. Furthermore, we thank Mrs. M. Apel and Mrs. U. Schmidt (Pharmacentrum Frankfurt) and Mrs. I. Rüter (Institute for Clinical Pharmacology and Pharmacology, HHU Düsseldorf) for excellent technical assistance. This work was supported by the ‘‘Forschungskommission der Heinrich-Heine-Universität’’ and by the Bayer AG, Wuppertal, Germany.

References

Blume, C., Heise, G., Mühlfeld, A., Bach, D., Schrör, K., Gerhardz, C.D., Grabensee, B., Heering, P., 1999. Effect of flosulide, a selective cyclooxygenase 2 inhibitor, on passive heymann nephritis in the rat. Kidney Int. 56, 1770–1778.

Brüne, B., 2002. Ntric oxide and apoptosis in mesangial cells. Kidney Int. 61, 786–789.

Cattell, V., 2002. Nitric oxide and glomerulonephritis. Kidney Int. 61, 816–821.

Chomczynski, P., Sacchi, N., 1987. Single step method of RNA isolation by acid guanidinium thiocyanate–phenolchloroform extraction. Anal. Biochem. 162, 156–159.

Cockerill, G.W., Rye, K.A., Gamble, J.R., Vada, M.A., Barter, M.J., 1995. High density lipoproteins inhibit cytokine induced expression of endothelial cell adhesion molecules. Atheroscler. Thromb. Vasc. Biol. 15, 1987–1994.

Danesh, F.R., Sadeghi, M.M., Amro, N., Philips, C., Zeng, L., Lin, S., Sahain, A., Kanwar, Y.S., 2002. 3-Hydroxy-3methylgloutaryl CoA reductase inhibitors prevent high glucose-induced proliferation on mesangial cells via modulation of Rho GTPas/p21 signalling pathway: implications for diabetic nephropathy. Proc. Natl. Acad. Sci. U. S. A. 99, 8301–8305.

Degraeve, F., Bolla, M., Blaie, S., Créminon, C., Quéré, I., Boquet, P., Lévy-Toledano, S., Bertoglios, J., Habib, A., 2001. Modulation of COX-2 expression by statins in human aortic smooth muscle cells. J. Biol. Chem. 276, 46849–46855.

Diaz-Cazorla, M., Perez-Sala, D., Lamas, S., 1999. Dual effect of nitric oxide donors on cyclooxygenase-2 expression in human mesangial cells. J. Am. Soc. Nephrol. 10, 943–952.

Furusu, A., Miyazaki, M., Abe, K., Tsukasaki, S., Shishita, K., Sasaki, O., Miyazaki, K., Ozono, Y., Koji, T., Harada, T., Sakai, H., Kohno, S., 1998. Expression of endothelial and inducible nitric oxide in human glomerulonephritis. Kidney Int. 53, 1750–1758.

Garg, U.C., Hassid, A., 1993. Mechanisms of nitrosothiol-induced antimitogenesis in aortic smooth muscle cells. Eur. J. Pharmacol. 237, 243–249.

Grandaliano, G., Biswas, P., Choudhury, C.G., Abboud, H.E., 1993. Simvastatin inhibits PDGF-induced DNA-synthesis in human mesangial cells. Kidney Int. 44, 503–508.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and (^{15}N) nitrate in biological fluids. Anal. Biochem. 126, 131–138.

Guijarro, C., Keane, W.F., 1996. Effects of lipids on the pathogenesis of progressive renal failure. Role of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in the prevention of glomerulosclerosis. Miner. Electrolyte Metab. 22, 147–152.

Haas, C., Schöcklmann, H.O., Lang, S., Kralewski, M., Sterzel, R.B., 1999. Regulatory mechanisms in glomerular mesangial cell proliferation. J. Nephrol. 12, 405–415.

Hattori, Y., Nakansishi, N., Kasai, K., 2002. Statin enhances cytokine-mediated induction of nitric oxide synthesis in vascular smooth muscle cells. Cardiovasc. Res. 54, 649–658.

Hausding, M., Witteck, A., Rodriguez-Pascual, F., von Eichel-Streiber, C., Forstermann, U., Kleinert, H., 2000. Inhibition of small G proteins of the rho family by statins or clostridium difficile toxin B enhances cytokine-mediated induction of NO synthase II. Br. J. Pharmacol. 131, 553–561.

Hernandéz-Perera, O., Pérez-Sala, D., Navarro-Antolin, J., Sanchez-Pascuala, R., Hernandéz, D., Díaz, C., Lamas, S., 1998. Effects of the 3-hydro-3-methylglutaryl-CoA-reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitrite oxide synthase in vascular endothelial cells. J. Clin. Invest. 101, 2711–2719.

Hruby, Z., Beck, K.F., 1997. Cytotoxic effect of autocrine and macrophage-derived nitric oxide on cultured rat mesangial cells. Clin. Exp. Immunol. 107, 76–82.

Huang, Y., Domann, F.E., 1998. Redox modulation of AP-2 DNA binding activity in vitro. Biochem. Biophys. Res. Commun. 249, 301–307.

Ishaque, A., Dunn, M.J., Sorokin, A., 2003. Cyccloxygenase-2 inhibits tumor necrosis factor alpha-mediated apoptosis in renal glomerular mesangial cells. J. Biol. Chem. 21, 10629–10640.

Kihara, M., Yabana, M., Kobayashi, S., Fujita, T., Iwamoto, T., Ishigami, T., Ishigami, T., Umemura, S., 1999. Angiotensin II inhibits interleukin 1-beta induced nitric oxide production in cultured rat mesangial cells. Kidney Int. 55, 1277–1283.

Kitahara, M., Eitner, F., Ostendorf, T., Kunter, U., Janssen, U., Westenfeld, R., Matsui, K., Kerjaschki, D., Floege, J., 2002. Selective cyclooxygenase-2 inhibition impairs glomerular capillary healing in experimental glomerulonephritis. J. Am. Soc. Nephrol. 13, 1261–1270.

Klein, T., Nusing, R.M., Pfeilschifter, J., 1994. Selective inhibition of cyclooxygenase 2. Biochem. Pharmacol. 18, 1605–1610.

Kunz, D.G, Walker, G., Pfeilschifter, J., 1994. Dexamethasone differentially affects interleukin-1β- and cAMP-induced nitric oxide synthase expression in rat mesangial cells. Biochem. J. 304, 3337–3340.

Kunz, D., Walker, G., Eberhardt, W., Pfeilschifter, J., 1996. Molecular mechanisms of dexamethasone inhibition of nitric oxide synthase expression in interleukin 1 beta stimulated mesangial cells: evidence for the involvement of transcriptional and posttranscriptional regulation. Proc. Natl. Acad. Sci. U. S. A. 93, 255–259.

Marnett, L.J., 2000. Cyclooxygenase mechanisms. Curr. Opin. Chem. Biol. 4, 545–552.

Mosmann, T.R., 1983. Rapid colorimetric assay for cellular growth and survival. application to proliferation and cytotoxicity assays. J. Immunol. Methods 16, 55–63.

Mühl, H., Pfeilschifter, J., 1995. Amplification of nitric oxide synthase expression by nitric oxide in interleukin 1 beta-stimulated rat mesangial cells. J. Clin. Invest. 95, 1941–1946.

Mühl, H., Sandau, K., Brune, B., Briner, V.A., Pfeilschifter, J., 1996. Nitric oxide donors induce apoptosis in glomerular mesangial cells, epithelial cells and endothelial cells. Eur. J. Pharmacol. 317, 137–149.

Nagasawa, K., Muraki, Y., Matsuda, T., Ohnishi, N., Yokoyama, T., 2000. Inhibitory effects of statins on fetal bovine serum-induced proliferation of rat cultured mesangial cells and correlation between their inhibitory effect and transport characteristics. J. Pharm. Sci. 89, 1594–1604.

Nogaki, F., Muso, E., Yashiro, M., Kasuno, K., Kamata, T., Ono, T., Sasayama, S., 1999. Direct inhibitory effects of simvastatin on matrix accumulation in cultured murine mesangial cells. Kidney Int. 71, 198–201.

Noris, M., Remuzzi, G., 1999. Physiology and pathophysiology of nitric oxide in chronic renal disease. Proc. Assoc. Am. Physicians 111, 602–610.

Oda, H., Keane, W.F., 1999. Recent advances in statins and the kidney. Kidney Inter., Suppl. 71, S2–S5.

O'Donnell, M.P., Kasiske, B.L., Kim, Yl., Atluru, D., Keane, W.F., 1993. Lovastatin inhibits proliferation of rat mesangial cells. J. Clin. Invest. 91, 83–87.

Park, J.K., Müller, D.N., Meervala, E.M.A., Dechend, R., Fiebeler, A., Schmidt, F., Bieringer, M., Schäfer, O., Lindschau, C., Schneider, W., Ganten, D., Luft, F.C., Haller, H., 2000. Cerivastatin prevents angiotensin II-induced renal injury independent of blood pressure and cholesterol-lowering effects. Kidney Int. 58, 1420–1430.

Pfeilschifter, J., Schwarzenbach, H., 1990. Interleukin 1 and tumor necrosis factor stimulate cGMP formation in rat renal mesangial cells. FEBS Lett. 29, 185–187.

Pfeilschifter, J., Schalkwijk, C., Briner, V.A., van den Bosch, H., 1993. Cytokine-stimulated secretion of group II phospholipase A_2 by rat mesangial cells. Its contribution to arachidonic acid release and prostaglandin synthesis by cultured rat glomerular cells. J. Clin. Invest. 92, 2516–2523.

Radeke, H.H., Mere, B., Topley, N., Floege, J., Habermehl, G.G., Resch, K., 1990. Interleukin 1-alpha and tumor necrosis factor-alpha induce oxygen radical production in mesangial cells. Kidney Int. 37, 767–775.

Rupprecht, H.D., Akagi, Y., Keil, A., Hofer, G., 2000. Nitric oxide inhibits growth of glomerular cells: role of the transcription factor EGR-1. Kidney Int. 57, 70–82.

Schrör, K., Seidel, H., 1988. Blood-vessel wall arachidonate metabolism and its pharmacological modification in a new in vitro assay system. Naunyn-Schmiedebergs Arch. Pharmacol. 337, 177–182.

Selden, J.A., Dolbeare, F., Clair, J.H., Nichols, W.W., Miller, J.E., Kleemeyer, K.M., Hylands, R.J., DeLuca, J.G., 1993. Statistical confirmation that immunofluorescent detection of DNA repair in human fibroblasts by measurement of bromodesoxyuridine incorporation is stoichometric and sensitive. Cytometry 14, 154–167.

Stahl, R.A., Thaiss, F., Haberstroh, U., Kahf, S., Shaw, A., Schoeppe, W., 1990. Cylooxygenase inhibition enhances rat interleukin 1 beta-induced growth of rat mesangial cells in culture. Am. J. Physiol. 259, F419–F424.

Stamatakis, K., Cernuda-Morollón, E., Hernandéz-Perera, O., Pérez-Sala, D., 2002. Isoprenylation of RhoB is necessary for its degradation. J. Biol. Chem. 51, 49389–49396.

Suyama, K., Kabuyama, Y., Suzuki, S., Kawasaki, Y., Suzuki, J., Suzuki, H., Homma, Y., 2001. Induction of transcriptional factor AP-2 by cytokines and prostaglandins in cultured mesangial cells. Am. J. Nephrol. 21, 307–314.

Tateyama, F., Yamabe, H., Osawa, H., Kaizuki, M., Shirato, K., Okumura, K., 2001. Interleukin-1beta is an autocrine growth factor of rat glomerular cells in culture. Nephrol. Dial. Transplant. 16, 1149–1155.

Tesch, G.H., Lan, H.Y., Atkins, R.C., Nikolic-Paterson, D.J., 1997. Role of interleukin-1β in mesangial cell proliferation and matrix deposition in experimental mesangioproliferative glomerulonephritis. Am. J. Pathol. 151, 141–150.

Tetsuka, T., Daphna-Iken, D., Miller, B.W., Guan, Z., Baier, L.D., Morrison, A.R., 1996. Nitric oxide amplifies interleukin 1-induced cyclooxygenase-2 expression in rat mesangial cells. J. Clin. Invest. 97, 2051–2056.

Westenfeld, R., Gawlik, A., de Heer, E., Kitahara, M., Abou-Rebyeh, F., Floege, J., Ketteler, M., 2002. Selective inhibition of inducible nitric oxide synthase enhances intraglomerular coagulation in chronic anti-Thy1-nephritis. Kidney Int. 61, 834–838.

Yoshimura, A., Nemoto, T., Sugenoya, Y., Inui, K., Waranabe, S., Inoue, Y., Sharuf, S., Yokota, N., Uda, S., Morita, H., Ideura, T., 1999. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. Kidney Int. 71, 84–87.