PERINDOPRILAT MODULATES THE ACTIVITY OF LIPOPROTEIN RECEPTOR-RELATED PROTEIN (LRP) IN HUMAN MESANGIAL CELLS.
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Running title: Perindoprilat modulates LRP expression.

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Low density lipoprotein related protein (LRP) is a multifunctional endocytic receptor implicated in the modulation of a number of cellular processes including the turnover of proteases and the degradation of extracellular matrix (ECM) proteins. As such it can play a key role in the control of fibrosis. The aim of the current investigation is to ascertain whether the anti-fibrotic effects exerted by the angiotensin converting enzyme inhibitor (ACE-I) perindoprilat on macrophage conditioned medium (MPCM)-injured human mesangial cells could be modulated by this receptor.

Addition of receptor associated protein (RAP) to MPCM-injured mesangial cells ±ACE inhibitor increased the amount of tPA protein detected in mesangial cell culture supernatants while not affecting the protein levels of plasminogen activator inhibitor (PAI-1). The ability of ACE-I to reduce fibronectin was diminished in the presence of RAP. ACE-I induced an increase in mesangial cell MMP9 mRNA but reduced the MMP9 enzyme activity detected in mesangial cell supernatants. Mesangial cell lysates from ACE-I treated cells were able to bind immobilised fibronectin at higher dilutions than cell lysates from untreated cells. Flow cytometry showed that MPCM induced an increase in LRP surface expression in mesangial cells over that of control and this expression was further increased by ACE-I treatment. The increase in LRP expression in response to ACE-I was also observed by Western blotting. Northern blot analysis on RNA extracted from cells following 24hr exposure to MPCM ± ACE-I demonstrated that there was no change in LRP mRNA expression on ACE-I treatment.

In conclusion we have shown that ACE-I treatment is able to modulate mesangial cell surface expression of LRP providing an additional mechanism whereby ACE-Is can mediate anti-fibrotic actions independent of their haemodynamic actions.
and LRP expression, and the subsequent events, which lead to observed reduction in fibronectin levels in human mesangial cells.

**Experimental procedures**

**Cell culture** - Human mesangial cells were cultured from the glomerular explants processed from the normal poles of nephrectomised human kidneys with renal carcinoma using standard serial sieving techniques [4]. All donors had given consent for post-surgical use of their kidneys and procedures on donated kidneys had been approved by ethical committee. The cells were cultured in RPMI 1640 (Invitrogen, Paisley, UK) supplemented with 20% heat inactivated fetal calf serum (FCS) (Sigma, Dorset, UK), 5μg/ml bovine insulin (Sigma), 100U/ml penicillin (Invitrogen), 100μg/ml streptomycin (Invitrogen), and 2mM glutamine (Invitrogen). Mesangial cells of passage 2 through 10 were cultured in 75cm² flasks (Costar-Corning, Buckinghamshire, UK). For experiments mesangial cells were grown to confluence in 24-well plates (Costar Corning) or 25cm² flasks (Costar-Corning) and then rendered quiescent in RPMI medium containing 0.5% FCS for 48 h prior to use. All experiments were carried out in RPMI +0.5% FCS.

Cells of the human monocyte/macrophage cell line U937 (ECCAC no. 85011440) were grown in RPMI 1640 supplemented with 10% FCS, 100U/ml penicillin, 100μg/ml streptomycin and 2mM glutamine.

**Preparation of U937 cell conditioned medium** - U937 cell conditioned medium was prepared as previously described [4].

**Preparation of cell lysates** - After removal of tissue culture supernatants, cell monolayers were washed with PBS, scraped into 250μl 1% IGEPAL CA-630 (Sigma) in wash buffer (PBS containing 0.3M NaCl and 1% Tween 20), and then incubated at room temperature for approximately 30 min. The cell scrapings were transferred to 2ml tubes, sonicated with a 5s burst and centrifuged for 1 min at 11,000 x g. Sonication and centrifugation were repeated, and the lysate supernatants were assayed for total cell protein.

**Culture of mesangial cells in the presence of macrophage conditioned medium (MPCM)** - Confluent, quiescent mesangial cells were exposed to a 50% solution of MPCM in the presence or absence 40μM of the ACE-I perindoprilat (gift from Servier, France) or in the presence or absence of 500nM receptor associated protein (RAP)(Calbiochem, Nottingham, UK). The cultures were maintained in the various conditions for 1 or 3 days. The tissue culture supernatants were harvested and stored at -20°C for subsequent analysis.

For Northern analysis mesangial cells were exposed to 50% MPCM ± additions for approximately 18 h prior to RNA processing.

**Fibronectin ELISA** - Culture supernatants were assayed for fibronectin as previously described [4].

**LRP Binding assay** - Assay was adapted from the protocol described by Salicioni et al [3]. 100μl of 10μg/ml human plasma fibronectin (Calbiochem) was immobilised onto 96 well microtitre plates (Costar-Corning) overnight at 4°C. 10μg/ml BSA (Sigma) was coated onto parallel plates to serve as a negative control. Non-specific binding sites blocked with 2% (w/v) BSA for 1 h. Cell lysates from mesangial cells exposed to MPCM± ACE-I or medium alone were added to the wells at dilutions of 1:10, 1:50 and 1:100 and incubated for 2 h. In parallel cell lysates were also incubated in the presence or absence of purified 4nM alpha-2 macroglobulin (α2mac)(Serotec, Oxford, UK). After washing the plates any LRP in the lysates that bound to immobilised fibronectin was detected using an anti-CD91 mouse monoclonal antibody (Serotec) at a 1:100 and incubated for 2 h. Bound primary antibody was detected with horseradish peroxidase labelled anti-mouse IgG antibody (DakoCytomation, Ely, UK) at a dilution of 1:1000 and incubated for 1 h. OPD (DakoCytomation) was used as the chromogenic substrate as described for the fibronectin ELISA [4].

**Flow cytometry** - Confluent, quiescent mesangial cells were stimulated with MPCM ± ACE-I for 18 h. Cell monolayers were washed in PBS containing 0.1% BSA and 0.05% sodium azide (PBA) after which the cells were detached from the flask surface using trypsin-EDTA (PBA) and then incubated at room temperature for approximately 30 min. The cell scrapings were transferred to 2ml tubes, sonicated with a 5s burst and centrifuged for 1 min at 11,000 x g. Sonication and centrifugation were repeated, and the lysate supernatants were assayed for total cell protein.

**Western Blotting** - Cell monolayers from each well were scraped into 150μl non-reducing
sample buffer. The samples were boiled for 5 min and resolved on 7% SDS-polyacrylamide gels. The gels were blotted onto nitrocellulose membranes and immunostained. Briefly, the membranes were blocked with a 2% solution of BSA in TTBS (TBS containing 0.5% Tween 20) for 1 hr. The membranes were then washed once in TTBS prior to incubation with anti-LRP antibody (1:100), for at least 2 hr at room temperature. After 3 more washes the membranes were incubated with alkaline phosphatase-labelled rabbit anti mouse immunoglobulins for at least 2 hr at room temperature. The membranes were washed a further 3 times before addition of chromogenic substrate (SigmaFast™ BCIP/NBT tablets).

**Protein Determination** - The protein content of cell lysates dissolved in 1% IGEPAI was determined using a commercial BioRad DC protein assay (BioRad, UK) using BSA standards according to the manufacturer’s instructions.

**Gelatin Zymography** - Culture supernatants were mixed 1:1 with non-denaturing sample buffer and resolved at 4°C on 8% SDS-polyacrylamide gels containing 2.5 mg/ml gelatin (electrophoresis grade, 300 bloom) (Sigma). The gels were washed in 2.5% Triton X 100 (Sigma) for approximately 1 hr after which they were incubated overnight at 37°C in pH7.6 developer for approximately 1 h after which they were washed in 2.5% Triton X 100 (Sigma). The polyacrylamide gels containing 2.5 mg/ml gelatin buffer and resolved at 4°C on 8% SDS-were mixed 1:1 with non-denaturing sample buffer. The samples were boiled for 5 min in 1% IGEPAL was further 3 times before addition of chromogenic substrate (SigmaFast™ BCIP/NBT tablets).

**Gelatin Zymography** - Culture supernatants were mixed 1:1 with non-denaturing sample buffer and resolved at 4°C on 8% SDS-polyacrylamide gels containing 2.5 mg/ml gelatin (electrophoresis grade, 300 bloom) (Sigma). The gels were washed in 2.5% Triton X 100 (Sigma) for approximately 1 hr after which they were incubated overnight at 37°C in pH7.6 developer buffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM CaCl₂, 0.05% (w/v) Brij). The gels were then stained with Brilliant Blue R® (Sigma) and destained with a solution of 40% methanol 10% acetic acid. Destained gels were dried and bands of lysis scanned using a BioRad imaging densitometer (model GS-700).

**Fibrinogen Zymography** - Culture supernatants were mixed 1:1 with non-denaturing sample buffer and resolved at 4°C on 11% SDS-polyacrylamide gels containing 12 mg fibrinogen (Calbiochem), 10 U plasminogen (Calbiochem), 10 U thrombin (Calbiochem) per 10 ml gel. Following electrophoresis the gels were washed, incubated in developer and stained as described for gelatin zymography.

**Northern blotting** - Northern analysis was carried out using a method previously described [4].

**RT-PCR** - 0.5 μg aliquots of total RNA were reverse transcribed using AMV reverse transcription system (Promega, Southampton, UK) according to the manufacturer’s instructions. The resulting cDNA was amplified using ReddyMix™ PCR Mastermix (ABgene, Surrey, UK) and 50 pmol of specific sense and anti-sense primers. Thermocycling conditions were optimised for each primer pair. Amplicons were resolved by electrophoresis on 1% Tris-acetate EDTA (TAE)-agarose gels.

**Oligonucleotide primers** - Reverse transcription-polymerase chain reaction (RT-PCR) of RNA extracted from mesangial cells stimulated with MPCM was carried out using the following primers (custom made by Invitrogen): LRP sense 5'-CCT ACT GGA CGC TGA CTT TGC-3', antisense 5'-GGC CCC CCA TGT AGA GTG T-3' [6], MMP9 sense 5'-AAG TAC TGG CGA TTC TCT GAG GG-3', antisense 5'-GGC TTT CTC TCG GTA CTG GAA GAC-3' [7]. MMP2 sense 5'TTT TCT CGA ATC CAT GAT GG-3', antisense 5'-CTG TG CAG CTC TCA TAT TT-3' [8].

**ELISA assays** - Imulysé™ tPA ELISA and TintElize™ PAI-1 ELISA (Alpha Laboratories, Hampshire, UK) were carried out according to manufacturer’s instructions.

**Statistics** - Mesangial cell fibronectin levels were corrected for cell protein. Fibronectin levels have been expressed as a percentage of levels obtained with MPCM. Representative autoradiographs of Northern blots, photographs of agarose gels or zymograms are shown but densitometric analysis incorporates data from all experiments. Results are expressed as means ± sem. For comparison of means between two groups, an unpaired t-test was employed. To compare values between multiple groups, an analysis of variance (ANOVA) with Bonferroni correction was applied. Statistical significance was defined as P<0.05.

**RESULTS**

Receptor associated protein (RAP) is the most avid ligand of LRP [9] and as such binds to LRP at the expense of other ligands. In order to examine whether ACE-Is play a role in LRP-mediated binding or endocytosis of tPA, RAP (500 nM) was added to MPCM-injured cells in the presence or absence of the ACE-I perindoprilat. As previously observed [4] the culture supernatants of MPCM-injured cells treated with ACE-I contained lower tPA levels than those from non-treated cells. Addition of RAP resulted in the accumulation of tPA in mesangial cell supernatants approximately doubling levels compared to non RAP treated cells. (Fig 1a) Supernatant tPA levels were typically 8.37±0.82 ng/ml (0.1 ng/μg cell protein) in MPCM injured cells and 1.63±0.17 ng/ml (0.012 ng/μg cell protein) in control cells while cell lysate tPA levels were typically 27.1±4.4 ng/ml (0.32 ng/μg cell protein) in injured cells and 6.39±1.1 ng/ml (0.07 ng/μg cell protein) in control cells. RAP did not have the same cumulative effects on PAI-1 levels. However,
RAP did reverse the perindoprilat-induced PAI-1 lowering effects which we have previously reported [4] (MPCM 2.81±0.05; MPCM+P 2.44±0.05; MPCM+RAP 2.81±0.04; MPCM+P+RAP 2.9±0.2; med 2.4±0.18ng/µg protein (n=2)).

The tPA assay data were supported by fibrinogen gel zymography on culture supernatants, which showed an accumulation of tPA activity in the presence of RAP (Fig 2). Mesangial cell lysates were analysed for the presence of immunoreactive tPA to assess whether the apparent ACE-I-induced reduction in tPA levels from culture supernatants could be accounted for by binding to, or uptake by, mesangial cells. The results demonstrated that tPA levels in the lysates from cells treated with ACE-I were higher than those in non-treated cells (Fig 1b) indicating that uptake of tPA by mesangial cells was enhanced in the presence of ACE-I. As observed in the supernatants, lysate tPA levels were also higher in RAP treated cells than cells treated with MPCM alone. However, there was no significant difference in lysate tPA levels between ACE-I treated cells regardless of whether they had been treated with RAP or not.

The ability of perindoprilat to decrease MPCM-injured mesangial cell fibronectin levels was diminished in the presence of RAP, although RAP itself appeared to have no effect on the fibronectin levels in non-ACE-I treated cells (Fig 3). The fact that fibronectin levels were not further reduced in the presence of accumulated tPA would suggest that tPA itself was not directly responsible for the observed fibronectin degradation as a result of its own protease activity or indirectly via plasmin and the subsequent activation of zymogens such as MMP2. Rather, it suggests that uptake of tPA results in the degradation of fibronectin perhaps via a mechanism downstream of tPA binding. It is possible that RAP-mediated inhibition of tPA binding results in reduced receptor signalling and diminution of the consequent downstream events which ultimately result in fibronectin degradation. We have previously observed (unpublished data) that addition of exogenous tPA (100ng/ml = 40u/ml = 1.5nM) to MPCM-stimulated mesangial cells resulted in a 15.34±2.1% reduction in MPCM-induced fibronectin levels. In the current study the levels of accumulated tPA were of the order of 20ng/ml and therefore probably insufficient to induce observable fibronectin degradation.

Tissue plasminogen activator is known to signal, as a cytokine via LRP causing increased transcription of MMP9 [10,11]. RT-PCR was carried out to assess mesangial cell expression of MMP9 and MMP2 mRNA in response to ACE-I treatment. RT-PCR demonstrated that MMP9 mRNA levels were upregulated in response to ACE-I (MPCM 0.662±0.105, MPCM+ACE-I *1.35±0.27, med 0.61±0.2 arbitrary densitometric units, *p<0.03 vs MPCM, n=4) while MMP2 mRNA levels appeared unaffected (Fig 4a). However, gelatin zymography on culture supernatants from MPCM-injured, ACE-I treated mesangial cells unexpectedly showed lower MMP9 activity than supernatants from non-ACE inhibitor treated cells (reduction in the active form of MMP9 was particularly pronounced)(Fig 4b). Co-incubation of MPCM-injured mesangial cells ± ACE-I in the presence of RAP was able to at least restore ACE-I-reduced MMP9 activity (Fig 4b). Since LRP can also function as a scavenger for MMP9 clearance [12], this may explain the observed reduction in MMP9 activity in supernatants from cells grown in the presence of ACE-I. In the presence of ACE-I active MMP2 enzyme levels were slightly increased. Treatment with RAP appeared to have no effect on MMP2 activity.

In order to confirm the role played by LRP in the anti-fibrotic actions of ACE-I we wished to assess whether ACE-I treatment affected the surface expression of LRP. LRP is known to have a fibronectin-binding domain within its structure [3]. This property of LRP was exploited to see whether increased levels of LRP could be detected in mesangial cell lysates following ACE-I treatment. Our data demonstrated that lysates from ACE-I treated cells were able to bind to immobilised fibronectin at higher dilutions than lysates from non-ACE-I treated cells (at the 1/50 dilution lysates from ACE-I treated cells bound significantly more fibronectin (p<0.03) than the same dilution in non-ACE-I treated cells. However, at the 1/100 dilution there was no significant difference in the binding between treatments)(Fig 5a). In the presence of α-2-macroglobulin, which competes for binding to LRP, this dilution effect was attenuated (Fig 5b).

Flow cytometry using antibodies to LRP demonstrated that MPCM-injured cells bound 37% more LRP antibody than control cells but cells treated with ACE-I bound 50% more than control cells (Fig 6a). Western blotting also reflected this small increase in LRP protein expression in response to ACE-I treatment (Fig 6b).

Northern blotting carried out on RNA extracted from cells following 24hr exposure to MPCM in the presence or absence of ACE-I...
demonstrated that there was no change in LRP mRNA message expression at this time point, possibly suggesting that the ACE-I exerted its effect at the level of protein expression (Fig 7).

DISCUSSION

Until very recently tPA had been regarded as an important component of the plasminogen activator system involved in the attenuation of extracellular matrix accumulation leading to the reduction of fibrosis after renal injury. Indeed it was in such a capacity that we tentatively ascribed the role of tPA when we demonstrated that the anti-fibrotic effects of ACE-I in mesangial cells were modulated via the bradykinin / plasminogen activator system axis [4]. A number of studies have shown that the role of tPA is far more complex and as a result, the functional role of tPA has been dramatically revised. It is now believed that tPA also plays an important, plasmin-independent, stimulatory, cytokine-like role responsible for a diverse number of physiological functions including the regulation of endothelial cell proliferation [14], modulation of neuron apoptosis [15] and transcriptional up regulation of MMP9 [10,11]. It must be said, however, that tPA’s multifarious plasmin dependent and independent functions are not mutually exclusive.

LRP, amongst its many functions, is able to control the ‘fibrotic status’ of its immediate environment. It is involved in the catabolism of extracellular matrix protein like fibronectin [3] as well as the homeostasis of proteinases like tPA and MMP9 [1]. LRP deficient murine embryonic fibroblasts have been shown to exhibit increased levels of cell surface fibronectin without the biosynthesis of the matrix protein having been altered [3]. Similarly antagonism of LRP with RAP has been shown to increase fibronectin accumulation in wild type fibroblasts. Transfection of full length LRP into deficient cells has also been shown to decrease fibronectin levels [3]. The current study has demonstrated that treatment with ACE-I results in altered LRP expression, which may have occurred post-transcriptionally since no changes in LRP mRNA levels were observed 24hr post treatment. The apparent increase in surface expression of LRP as seen in the presence of MPCM compared to control (medium alone) may be attributed to a decreased rate of receptor cycling that can occur in the presence of an injurious agent [16], in this case MPCM. This phenomenon may also contribute to the observed accumulation of fibronectin in the presence of MPCM, as reduced cycling would ultimately delay fibronectin degradation. ACE-I treatment may therefore facilitate an increase in LRP cycling rates or alter the subcellular distribution such that receptors are more rapidly translocated from the endoplasmic reticulum to the surface of the cell such as has been found previously in vascular smooth muscle cells following treatment with epidermal growth factor and platelet derived growth factor [16].

Studies have shown that treatment of murine fibroblasts with RAP causes an accumulation of MMP9 due to inhibition of LRP’s ability to scavenge MMP9 [11]. In the current study we did not observe an accumulation of MMP9 in the presence of RAP but rather an accumulation of tPA, suggesting that in our system RAP was blocking the binding and endocytosis of tPA and thereby the subsequent signalling required to up regulate the expression of MMP9. We could speculate that these increased levels of intracellular tPA could be the consequence of a RAP-induced deceleration of the receptor cycling process resulting not only in an accumulation of extracellular tPA but also increased intracellular tPA as a result of a reduced rate of tPA degradation.

We observed a paradoxical decrease in MMP9 activity in ACE-I treated, MPCM-injured mesangial cells, which was at least restored on treatment with RAP. This suggests that increased LRP surface expression as well as resulting in increased MMP9 mRNA expression may also be responsible for an increased rate of MMP9 protein being scavenged from the culture supernatants. This observation suggests that a dynamic balance exists between tPA binding and signalling leading to the up regulation of MMP9 expression, and tPA and MMP9 endocytosis leading to clearance of the proteases via the same receptor. MMP2 mRNA levels did not appear to be affected by ACE-I treatment. The change in enzyme activity therefore probably represents a post-translational change in preformed zymogen levels.

Whether the ACE-I-mediated effects on LRP expression are the result of a direct action of ACE-I molecules on LRP or are the consequent down stream effects of ACE-I-induced tPA acting as a cytokine via LRP remains to be determined.

The dose of 40μM perindoprilat used in the current study was based upon a dose response curve carried out for a previous study [4]. This equates to a concentration of 14.7μg/ml which is higher than the peak plasma concentration (Cmax) of either perindopril (prodrug) or
perindoprilat typically observed following a single 4mg dose, in a human subject (64ng/ml and 4.7mg/ml respectively)[18], (although patients can receive up to 8mg/day). However, direct comparisons between in vivo and in vitro are problematic since the pharmacokinetics of a drug in cell culture will be different to those of the same drug in a human subject. Moreover, patients are dosed daily over long periods of time while cells in the current study were exposed to a single dose for 1 or 3 days. Whether the observed in vitro effects also occur in human patients receiving perindopril is still to be fully determined. However, the study has identified LRP as a possible target of ACE-I action and provides a platform for future research.

In conclusion, we have demonstrated for the first time that ACE-I treatment results in the modulation of mesangial cell surface expression of LRP, potentially allowing for increased fibronectin catabolism, and tPA binding and signalling – beneficial anti-fibrotic effects that are independent of ACE-I haemodynamic actions.

REFERENCES


FOOTNOTES

Parts of this manuscript were presented in abstract form at the annual American Society of Nephrology meeting in San Diego 2006.
The abbreviations used are: LRP, Low density lipoprotein receptor related protein; RAP, receptor associated protein; macrophage conditioned medium, MPCM; ACE-I, angiotensin converting enzyme inhibitor; α2mac, alpha-2 macroglobulin.

FIGURE LEGENDS

**Fig 1.** Effect of RAP on ACE inhibitor-treated mesangial cell tPA levels. Mesangial cells were treated in the presence or absence of ACE-I, and/or presence or absence of RAP. Culture supernatants and cell lysates were analysed for tPA by ELISA. (A) Supernatants (B) Cell lysates. Results show means ± sem (*p= 0.002 vs MPCM, #p<0.02 vs MPCM and MPCM+ACE-I n=4, **p=0.03 vs MPCM, †p<0.04 vs MPCM, NS between MPCM and MPCM+RAP or MPCM+ACE-I+RAP n=4).

**Fig 2.** Effect of RAP and ACE inhibitor treatment on tPA activity. Culture supernatants from mesangial cells treated in the presence or absence of ACE-I and/or in the presence or absence of RAP were analysed for active tPA by fibrinogen zymography. A representative zymogram from 4 experiments is shown.

**Fig 3.** Effect of RAP on supernatant fibronectin levels. Supernatants from cells treated in the presence or absence of ACE-I and/or the presence or absence of RAP were analysed for fibronectin using an inhibition ELISA. Results show means ± sem (*p=0.002 vs MPCM, n=5, NS between MPCM vs MPCM+RAP and MPCM+RAP+ACE-I).

**Fig 4.** Effect of ACE inhibitor treatment on MMP9 and MMP2 mRNA expression. RT-PCR was carried out on RNA extracted from cells treated in the presence or absence of ACE-I. A Agarose gel shows PCR amplification products of MMP9 and MMP2 following treatment. Representative gel from 3 experiments. Gelatin zymography was carried out on culture supernatants from cells treated in the presence or absence of ACE-I and the presence or absence of RAP. B Gelatin zymogram shows MMP9 and MMP2 pro and active enzyme activity following treatment. Representative zymogram from 3 experiments.

**Fig 5.** Binding to immobilised fibronectin. Dilutions of cell lysates obtained from mesangial cells treated in the presence or absence of ACE-I were examined for their ability to bind immobilised fibronectin. Binding assays were carried out in the absence (a) or presence (b) of alpha 2 macroglobulin. Results show mean± sem (*p<0.03 vs 1/50 dilution of MPCM lysates or medium alone lysates, n=4 (ANOVA). There was no significant difference in fibronectin binding of lysates at the1/100 dilution). There was no significant difference in binding between treatments (±ACE-I) in the presence of alpha 2 macroglobulin.
Fig 6a. Detection of expression of LRP by flow cytometry and Western blotting.
a. Mesangial cells treated in the presence or absence of ACE-I were analysed for cell surface expression of LRP using the binding of anti-LRP antibody. The results are expressed as mean ± sem of arbitrary mean cell fluorescence units (*p<0.03 vs MPCM, **p<0.02 vs medium, n=8)
b. Lysates from mesangial cells treated in the presence or absence of ACE-I were resolved by SDS-PAGE, western blotted onto nitrocellulose membranes and immunostained with anti LRP antibody. A representative blot from 3 experiments is shown.

Fig 7. Effect of ACE-I on LRP mRNA expression. RNA from mesangial cells treated in the presence or absence of ACE-I were analysed by Northern blotting. Autoradiograph shows representative Northern blot from 4 experiments.
Figure 1

A Supernatants

B Cell Lysates
Figure 2

kD

82
44
30

MPCM  MPCM  MPCM  MPCM  Med
+ACE-I  + RAP  + RAP  + RAP  + ACE-I
Figure 3

Fibronectin % of MPCM

- MPCM
- MPCM + ACE-I
- MPCM + RAP
- MPCM + RAP + ACE-I
- Med
Figure 4.

A

MMP9

MMP2

GAPDH

MPCM MPCM Med
+ACE-I

B

Pro MMP9 →
Active MMP9 →
Pro MMP2 →
Active MMP2 →

MPCM MPCM MPCM MPCM Med
+ACE-I +RAP +RAP +ACE-I
Figure 5.

A

Fibronectin binding (Arbitrary OD units/\(\mu\)g protein)

B

Cell lysate dilutions
Figure 6

A

![Bar chart showing arbitrary fluorescence units for MPCM, MPCM + ACE-I, and Med conditions.](chart.png)

B

![Image showing LRP bands for different conditions: MPCM, MPCM + ACE-I, MPCM + RAP, MPCM + ACE-I + RAP, and Med.](image.png)
Figure 7

LRP

cyclophilin

MPCM  MPCM +ACE-I  Med