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Procalcitonin induced cytotoxicity and apoptosis in mesangial cells: implications for septic renal injury

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Abstract
Objective and design Immuno-neutralization of procalcitonin (ProCT) has been shown to ameliorate experimental sepsis as well as the renal complications of this disease. Accordingly, we investigated the direct effect of ProCT on mesangial cells (MCs).
Material Primary culture of murine MCs.
Treatment ProCT (0.5, 1.0, 2.5, 5.0 ng/ml) for 2, 4, 6 h.
Methods MCs were exposed in vitro to ProCT. Expression levels of IL-6, iNOS and TNF-α were determined by real time RT-PCR, Inflammatory pathways, and a panel of cytokines and chemokines involved in the process were investigated by PCR array; apoptosis/viability were evaluated in a multiplex assay and actin cytoskeleton alterations were examined by immunofluorescence (IF).
Results ProCT caused an early elevation in both IL-6 and iNOS mRNA (2–4 h), and a later rise (6 h) in TNF-α mRNA. ProCT upregulated genes of proinflammatory pathways 5- to 24-fold compared to control. IF images revealed disruption of the actin cytoskeleton and retraction of cell bodies with loss of typical stellate or spindle shape phenotype. ProCT decreased MCs viability by 36 % compared to control cells and induced significant apoptosis.
Conclusions ProCT has direct cytotoxic properties and may play a role in septic acute kidney injury that is independent of endotoxemia or hemodynamic alterations.

Keywords Procalcitonin · Mesangial cells · Interleukin 6 · Inducible nitric oxide synthase · Apoptosis · Actin

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Introduction

Procalcitonin (ProCT) is the 116 aminoacid precursor of the hormone calcitonin. ProCT serum concentration is an important biomarker of the severity and prognosis of systemic infections [1]. Both experimental sepsis and exposure to lipopolysaccharide (LPS) resulted in a multifold elevation of ProCT arising from a diverse cellular origin, including the kidney [2–4]. In our previous studies with a porcine sepsis model, both early [5] and late [6] immuno-neutralization of ProCT significantly ameliorated systemic and renal complications, supporting a role for this prohormone as a biomediator of the systemic inflammatory response [1, 7–9].

The glomerular mesangial cell (MC) is a pericyte with a measurable role in renal function both in health and disease [10–14]. In vitro studies indicated that the creation of a

proinflammatory milieu that is similar to that unleashed by the injection of bacterial endotoxin altered the biological properties of MCs [15–19] resulting in abnormalities of glomerular function and structure. Whether ProCT directly affects MCs has not been studied.

In the present study, we evaluated the functional, biological and ultrastructural effects of ProCT on MCs. We hypothesized that, similar to vasoactive peptides and mediators of inflammation, ProCT exerts a direct effect on MCs through activation of proinflammatory genes, alterations of the actin cytoskeleton and induction of apoptosis.

Materials and methods

Cell culture

Murine MCs (primary cultures) were grown in a 3:1 mixture of DMEM and F12 medium containing 6 mM glucose, 1 mM glutamine, 0.075 % $NaHCO_3$, 100 U/mL penicillin/100 μg/mL streptomycin and 10–20 % fetal bovine serum (FBS). Upon reaching approximately 80 % confluence, cells were dispersed with trypsin/EDTA, and plated in cell culture dishes pre-coated with human fibronectin, at an adequate cell density for each experiment.

RNA extraction and real time RT-PCR

MCs were plated at a density of 20,000 cells/well into a six-well plate and grown with DMEM and 20 % FBS for 2 days. The medium was replaced by DMEM with 2 % FBS for 24 h and the cells were subsequently incubated with bioactive ProCT of human origin [9, 20]. Dose–response curves and time-course experiments were performed to determine the optimal ProCT dose and time of study. The cells were homogenized and total RNA was extracted using the RNeasy mini kit (Qiagen, Valencia, CA) following the manufacturer's protocol. RNA samples (100 ng) were employed in duplicate to determine a relative quantification of target transcript using real time RT-PCR with the TaqMan® One-Step RT-PCR Master Mix Reagents (Applied Biosystems, Foster City, CA) and the ABI 7500 Sequence Detector System (Applied Biosystems). Normalization was calculated by the difference between target and GAPDH C_T levels (ΔC_T). Primers and TaqMan® probes specific for TNF-α, IL-6 and iNOS genes were designed using Primer Express software (Applied Biosystems). Specificity of transcript amplification was verified using a nucleotide Basic Local Alignment Search Tool (BLAST, National Center for Biotechnology Information, NIH).

PCR array

MCs were grown in two T-25 flasks as previously described and incubated with or without ProCT (5 ng/mL) for 4 h. The cells were harvested and RNA was extracted and transcribed to cDNA with RT^2 first strand kit (SABiosciences/Qiagen, Maryland). A signal transduction pathway finder and cytokines/chemokines (SABiosciences/Qiagen, Maryland) PCR arrays were performed to build a panel of genes related to several transduction and inflammatory pathways affected by ProCT.

Immunofluorescence analysis (F-actin and α-smooth muscle actin, or α-SMA)

To assess ProCT-induced morphological changes, a qualitative study was performed by exposing MCs (2,000 cells/well) to ProCT (5 ng/mL) for 4 h and fixed in 4 % paraformaldehyde. Fixed cells were blocked with 1 % BSA, and incubated with Alexa Fluor 488 phalloidin (Invitrogen, Carlsbad, CA) or with mouse monoclonal anti-α-SMA antibody (Sigma-Aldrich, St Louis, MO) followed by goat anti-mouse Alexa 488 IgG (Invitrogen, Carlsbas, CA). Slides were mounted using ProLong Gold with DAPI (Invitrogen, Carlsbad, CA) and images were captured using a Nikon NIS-80i fluorescent microscope coupled to a digital camera.

Apoptosis assay

MCs were plated (2,000 cells/well) into a dark 96-well plate and grown with DMEM (without phenol red) as previously described. Apoptosis and cell viability were measured in the same sample with ApoLive-Glo Multiplex Assay (Promega, Madison, WI) 4 h after incubation with ProCT (2.5 and 5 ng/mL). In this assay, a substrate was used to measure the activity of caspases three and seven, which play a central role in the execution-phase of cell apoptosis. Cell viability was measured by a fluorogenic substrate to a live-cell protease, which is restricted to intact viable cells. Staurosporin (10 μM) was used as a positive control of apoptosis.

Statistical analysis

Each quantitative experiment was performed independently at least twice, with 2–5 replicates. For RT-PCR time–course curve, a non-paired t test was used to compare ProCT with respective time-controls. For all other comparisons, one-way ANOVA was used, followed by Student–Newman–Keuls test. Results are mean ± SEM. Statistical significance was defined as $p < 0.05$.

Results

Figure 1 shows mRNA transcript levels of IL-6, iNOS and TNF-α after incubation of MCs with increasing concentrations of ProCT (panels b, d, f) and a time-course study (panels a, c, and e). IL-6 mRNA was significantly and progressively increased after 2, 4 and 6 h of ProCT exposure (Fig. 1a). ProCT induced an increase of 40-fold in IL-6 mRNA at 2.5 ng/mL and approximately 350-fold at 5 ng/mL (Fig. 1b). ProCT also induced a 5- and 10-fold increase in iNOS mRNA at 2.5 ng/mL (Fig. 1d) and after 4 and 6 h (Fig. 1c) while TNF-α increased only after a 6 h incubation with 5 ng/mL of ProCT (Fig. 1e, f). Thus, MCs exposed to ProCT produced soluble mediators of inflammation, a phenomenon consistent with a direct cytotoxic effect.

Tables 1 and 2 show, respectively, a summary of the genes up- or downregulated by ProCT (5 ng/mL, for 4 h) in 18 different signal transduction pathways and in eight cytokine/

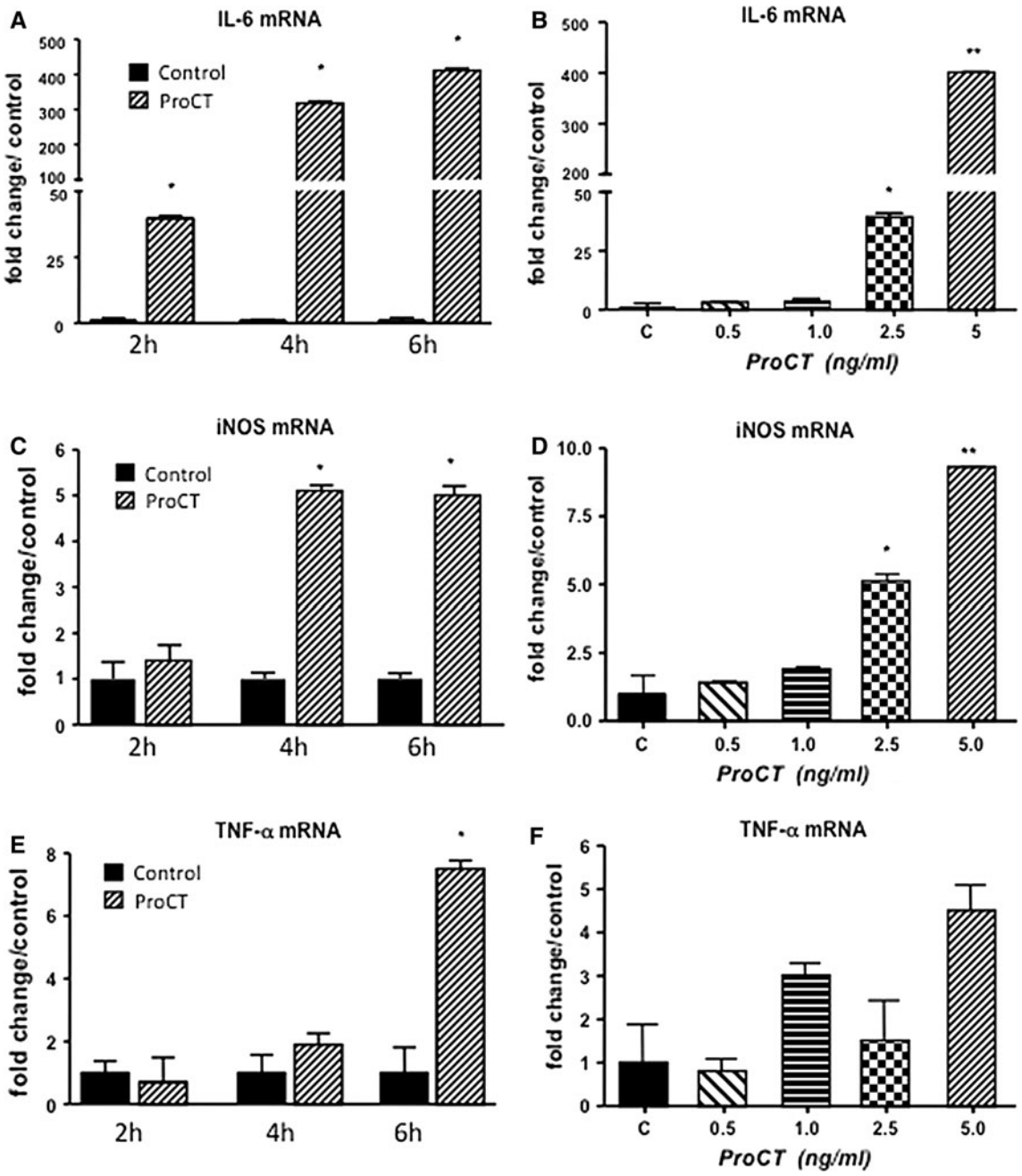

Fig. 1 mRNA transcript levels of IL-6, iNOS and TNF-α in cultured mesangial cells (MC) exposed to ProCT (5 ng/ml) for 2, 4 and 6 h (**a**, **c**, **e**) or to several ProCT concentrations (**b**, **d**, **f**) for 4 h. Each *graph* represents one of two experiments performed in quintuplicate. $*p < 0.01$ and $**p < 0.001$ vs control

Table 1 Pathway analysis of gene expression changes in MC exposed to ProCT (5 ng/ml) for 4 h

Expression profile of 84 genes representative of 18 different signal transduction pathways was performed (Supplemental Table S1)

The genes listed represent the significantly up- and downregulated functional categories analyzed with RT^2 Profiler™ PCR Array Data Analysis software (Qiagen/SABiosciences)

Pathway	Upregulated genes (>twofold)	Downregulated genes (<twofold)
Mithogenic	Egr1, Fos, Jun	
Wnt	Jun, Myc, Vegfa, Wisp1	Birc5, Pparg, Tcf7
Hedgehog	Wnt1, Wnt2	En1, Ptch1
p53	Gadd45a, Igfbp3, Mdm2	Ei24
Stress	Fos, Hspb1, Myc	
NFkB	Ccl20, Cxc11	Ikbkb, IL-1a
CREB	Egr1, Fos	
Jak–Stat	IL-4ra, Mmp10	Irf1
Estrogen	Igfbp4	Brca1
Calcium and PKC	Fos, Jun, Myc	
Phospholipase C	Fos, Jun, Ptgs	
Insulin	Cebpb, Hk2	Gys1
LDL	Ccl2, Csf2, Sele	
Retinoic acid		Hoxa1

Table 2 Chemokines/Cytokines expression changes in MC exposed to ProCT (5 ng/ml) for 4 h

	Upregulated genes (>twofold)	Downregulated genes (<twofold)
Chemokines	Ccl11, Ccl2, Ccl20, Ccl3, Ccl4, Ccl5, Ccl7, Cx3c11, Cxc11, Cxcl10, CxCl3, Cxcl5	
Interleukins	IL-10, IL-11, IL-17f, IL-1a, IL-1rn, IL-24, IL-27, IL-6	IL-3, IL-15, IL-11a
Growth factors	Csf1, Csf3, Lif, Vegfa	Bmp4
TNF superfamily	Tnfrsf11b	
Anti-inflammatory cytokines	IL-10, IL-11, IL-12b, IL-24, Tgfb2	IL-13

Expression profile of 84 genes representative of seven different gene groupings is listed in Supplemental Table S2

The gene expression analysis was performed with RT^2 Profiler™ PCR Array Data Analysis software (Qiagen/SABiosciences)

chemokine pathways (Supplemental Tables S1 and S2). Among a total of 84 genes analyzed in each PCR array, 26 genes involved in several signal transduction pathways were significantly upregulated (Table 1, Supplemental Table S1). The Wnt, p53, stress and Ca^{2+}/PKC pathways were more significantly affected by ProCT, with most of the genes increasing 5- to 24-fold compared to control ($p < 0.05$). Several elements involved in cell defense, such as PPAR, BIRC5, En1, and Ptch1 were downregulated by ProCT. Genes associated with cytokines, chemokines, and co-stimulatory molecules were mostly up-regulated by ProCT (Table 2). Some anti-inflammatory cytokines such as IL-10, IL-11, IL-12b and IL-24 were also upregulated, suggesting an attempt by the MC to counteract the cytotoxic effect of ProCT.

MCs exposed to ProCT for 4 and 6 h lost their confluence and showed signs of cytoplasmic and nuclear shrinkage (Fig. 2). MCs at rest displayed abundant parallel actin microfilaments running throughout the cytoplasm (Fig. 3a, c). ProCT induced cytoskeletal alterations and contraction of MCs (Fig. 3d) as compared to control (Fig. 3c). Following 4 h of exposure to ProCT, MCs displayed changes in cell shape, and no longer exhibited a discernible array of F-actin filaments but instead showed a retracted cytoplasm with less intense staining that was consistent with the loss of the typical actin distribution (Fig. 3b). More dramatic changes were visualized in α-SMA stained cells characterized by coalescing, nuclear fusion, and a loss of the typical cytoplasmic extensions (Fig. 3d).

As demonstrated in Fig. 4, ProCT (5 ng/mL) decreased MC viability by 36 % compared with control cells (black bars, $p < 0.001$), mainly due to the induction of apoptosis (114 % increase vs control, hatched bars, $p < 0.001$). At 2.5 ng/mL, ProCT decreased cell viability by 16 % and did not induce apoptosis. Staurosporin (10 μM) was used as a positive control for apoptosis. The combined measurement of apoptosis and cell viability used in this study provides an accurate distinction of apoptosis from other mechanisms of cell death. The ratio of caspase activity to viable cells is useful for determining the extent of caspase activation and for normalizing cell number.

Discussion

The principal finding of this study was a direct toxic effect of ProCT on MCs. Our previous studies had shown that

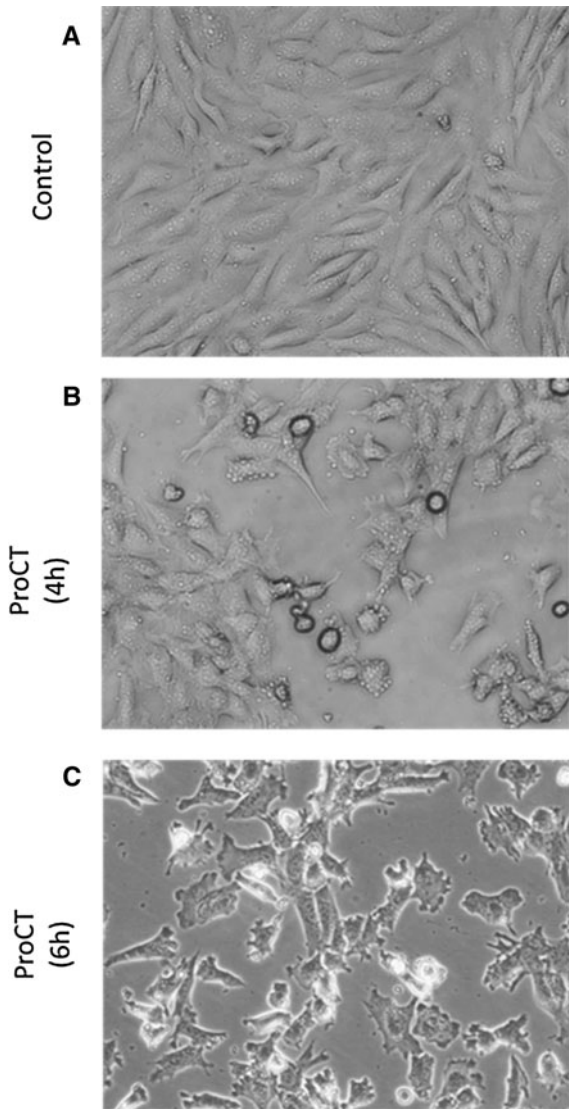

Fig. 2 Mesangial cells in 2 % FBS DMEM (**a**) maintained their spindle shape and confluence throughout the experiment. After 4 h incubation with ProCT (5 ng/mL) the cells lost confluence and displayed changes in shape (**b**). After 6 h incubation with ProCT, cells showed signs of cytoplasmic and nuclear shrinkage (**c**). Magnification ×10

blocking ProCT during the course of experimental sepsis in pigs mitigated the abnormal systemic hemodynamic parameters and renal function [5, 6]. The results obtained in the present study contribute to an elucidation of the mechanisms whereby neutralization of ProCT may be beneficial. MCs, recognized as playing an active role in both inflammatory and non-inflammatory renal injury [12], responded to ProCT by increasing synthesis of IL-6, iNOS, and TNF-α, enhanced expression of inflammatory genes and pathways, disruption of the actin microfilament network, and apoptosis. The murine MCs used in this study have been previously well characterized, and develop physiological and pathological characteristics that permit the study of human kidney diseases [21–24]. In this in vitro model, there is no appreciable conversion of ProCT into calcitonin. Previous studies using purified human ProCT in hamsters demonstrated a successful heterologous response, thus suggesting that the effects observed in murine MCs exposed to ProCT resulted from a specific hormone-receptor interaction. This hypothesis is supported by the fact that the homology between the human and mouse ProCT amino acid sequence is fairly high (77 %) [25]. Future studies to elucidate the interaction of calcitonin peptides with receptors in MCs and in other renal cells will provide an attractive interventional perspective for septic AKI.

Levels of iNOS were very low in normal MCs but increased dramatically after exposure to cytokine or LPS [26, 27]. An increased and prolonged production of NO by iNOS was associated with cytotoxicity via formation of iron-nitrosyl complexes and inactivation of iron-containing enzymes and through reaction with superoxide to generate toxic hydroxyl radicals [28]. Our studies show that iNOS is upregulated by ProCT in MCs, supporting the hypothesis that several renal alterations observed during sepsis and attributable to LPS, could be a direct consequence of ProCT effects.

Although TNF-α [29, 30] and IL-1β [29, 31] are usually the proinflammatory mediators of LPS in sepsis, in our study TNF-α was upregulated only after 6 h of ProCT incubation, a time-point when most of the MCs had reached advanced stages of apoptosis. These findings suggest that the cellular mechanisms triggered by ProCT may also involve distinct inflammatory pathways independently of LPS.

PCR arrays analysis revealed that several genes of the Wnt pathway were upregulated in MCs exposed to ProCT. Aberrant regulation of Wnt/β-catenin signaling pathway has been implicated in many types of chronic kidney diseases as well as in nephrotoxic acute kidney injury (AKI) [32–37]. The non-canonical Wnt signaling pathway has two intracellular signaling cascades that consist of the Wnt/Ca^{2+} pathway and the WnT/PCP pathway. The Wnt/Ca^{2+} pathway triggers cellular processes that involve activation of PLC, increase of $(Ca^{2+})_i$, decreased cGMP (cyclic guanosine monophosphate) levels, and activation of CamKII (Ca^{2+}—calmodulin-dependent protein kinase-II) or Caln (calcineurin) and PKC (protein kinase C) [32, 33]. These signals can then stimulate transcription factors like CREB (cAMP response element-binding protein-1). The Wnt/PCP pathway activates Rho/Rac small GTPase and JNK (Jun *N*-terminal Kinase) to assist in regulation of cytoskeletal organization and gene expression. Our findings that genes of PLC, Ca^{2+}/PKC, CREBS and LDL pathways were also upregulated in MCs suggest that activation of the Wnt pathway may underlie ProCT toxicity.

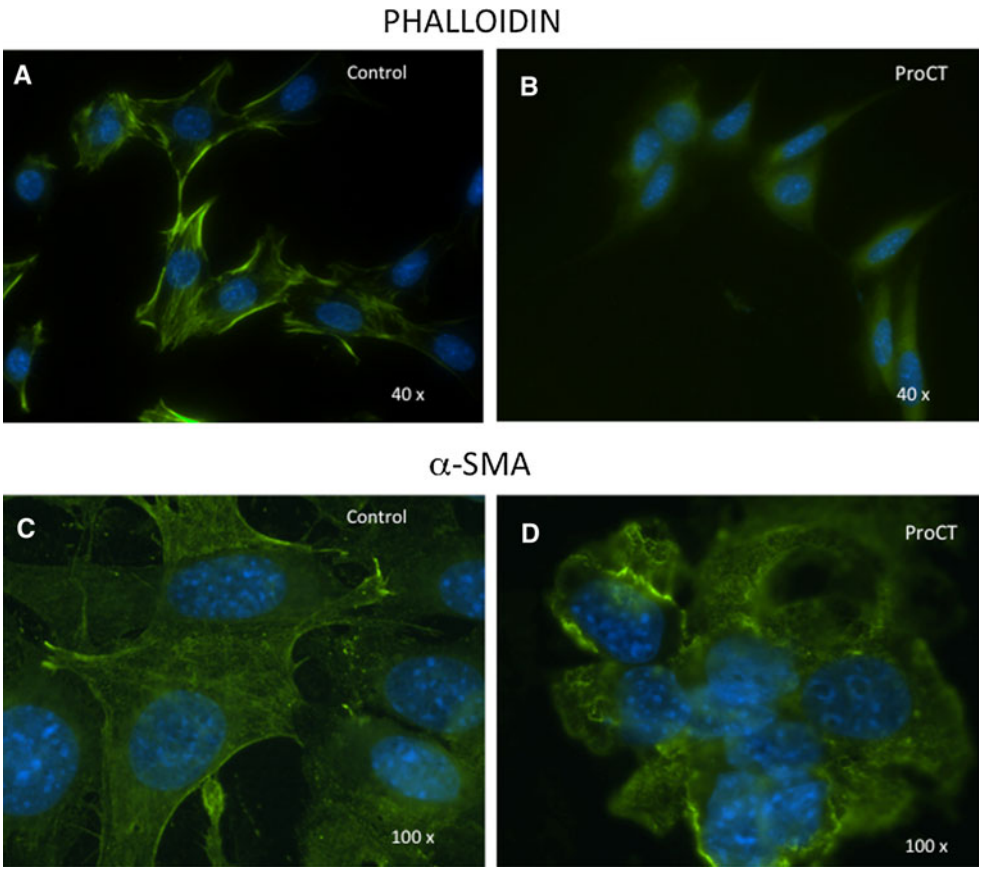

Fig. 3 Immunofluorescence staining of MC grown in chambers on glass slides and exposed or not to ProCT (5 ng/ml) for 4 h. The F-actin filaments of the cytoskeleton were stained with Alexa 488 Phalloidin (**a**, **b**) and the alpha smooth muscle actin with mouse monoclonal anti-α-SMA antibody (**c**, **d**). Both staining are shown in *green*; nuclei are stained in *blue* with DAPI

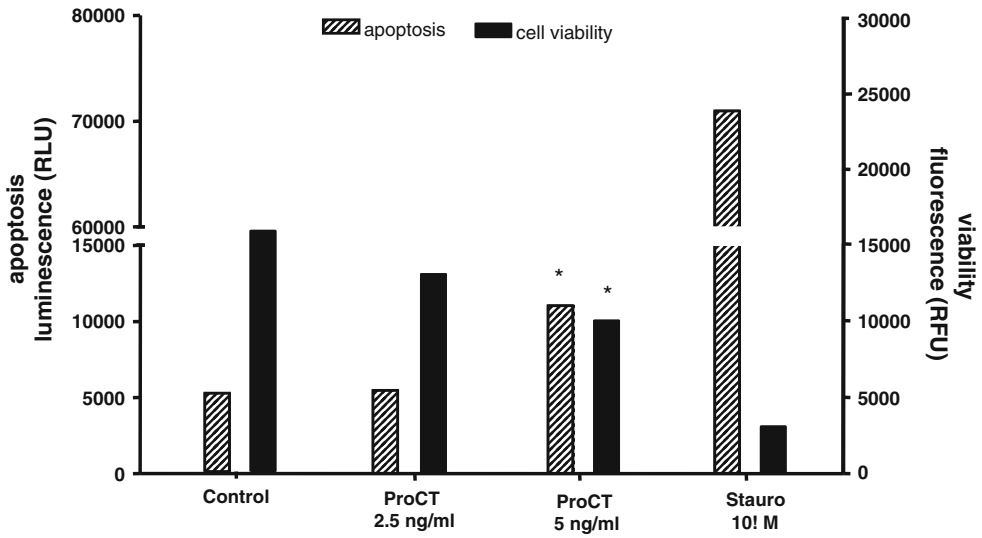

Fig. 4 Apoptosis (*dashed bars*) and cell viability (*black bars*) of cultured MCs exposed or not to ProCT (2.5 and 5 ng/ml) for 4 h. Staurosporin was used as a positive control for apoptosis. $^{*}p < 0.001$ vs control (ANOVA, Newman Keuls post test)

The upregulation of a vast number of genes associated with proinflammatory chemokines suggests that ProCT participates in the inflammatory response not only by stimulating local production of cytokines but also by triggering a chemotactic response that further augments its toxic effects.

MCs offer structural support to the glomerular capillary through sub-membranous microfilament bundles that are continuous with microtendinous projections tethered to the glomerular basement membrane. These microfilaments are ideally distributed to withstand tensional stress originating in the glomerular microcirculation [38]. The substantial cytoskeleton changes observed after incubation with ProCT suggest that MCs may be incapable of rendering tonic resistance to stretch, a phenomenon associated with the expression of certain inflammatory mediators such as

ICAM-1 [39] and this may also contribute to the early decline in the glomerular filtration rate of experimental sepsis that occurs despite the systemic hypotension [40, 41].

IL-6 has recently emerged as an important marker and/or mediator of AKI. In humans with sepsis, IL-6 levels strongly correlated with the incidence of AKI [42]. In a model of porcine sepsis, renal expression of ProCT positively correlated with IL-6 [4]. Therefore, the powerful stimulation of IL-6 production by MCs induced by ProCT is of potential relevance to septic AKI.

In summary, this study demonstrates direct cytotoxic effects of ProCT on MCs that are independent of bacterial endotoxemia and hemodynamic perturbations. These findings reflect the motive force of ProCT as a toxic mediator in sepsis-related AKI. The fact that mouse mesangial cells have been validated as an in vitro model for human kidney disease [21–24] and that our previous work in pigs [5, 6] demonstrated beneficial effects of immuno-neutralization of ProCT on the course of experimental sepsis makes our findings potentially relevant to humans.

References

1. Becker KL, Nylen ES, White JC, Muller B, Snider RH Jr. Clinical review 167: procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab. 2004;89:1512–25.
2. Morgenthaler NG, Struck J, Chancerelle Y, Weglohner W, Agay D, Bohuon C, et al. Production of procalcitonin (PCT) in non-thyroidal tissue after LPS injection. Horm Metab Res. 2003; 35:290–5.
3. Muller B, White JC, Nylen ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab. 2001;86: 396–404.
4. Zannoni A, Giunti M, Bernardini C, Gentilini F, Zaniboni A, Bacci ML, et al. Procalcitonin gene expression after LPS stimulation in the porcine animal model. Res Vet Sci. 2012;93:921–7.
5. Wagner KE, Martinez JM, Vath SD, Snider RH, Nylen ES, Becker KL, et al. Early immunoneutralization of calcitonin precursors attenuates the adverse physiologic response to sepsis in pigs. Crit Care Med. 2002;30:2313–21.
6. Martinez JM, Wagner KE, Snider RH, Nylen ES, Muller B, Sarani B, et al. Late immunoneutralization of procalcitonin arrests the progression of lethal porcine sepsis. Surg Infect. 2001;2:193–202 Discussion 202–3.
7. Becker KL, Snider R, Nylen ES. Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. Br J Pharmacol. 2010;159:253–64.
8. Nylen ES, Alarifi AA. Humoral markers of severity and prognosis of critical illness. Best Pract Res Clin Endocrinol Metab. 2001;15:553–73.
9. Nylen ES, Whang KT, Snider RH Jr, Steinwald PM, White JC, Becker KL. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Crit Care Med. 1998;26:1001–6.
10. Badr KF, Brenner BM, Ichikawa I. Effects of leukotriene D4 on glomerular dynamics in the rat. Am J Physiol. 1987;253:F239–43.
11. Iversen BM, Kvam FI, Matre K, Morkrid L, Horvei G, Bagchus W, et al. Effect of mesangiolysis on autoregulation of renal blood flow and glomerular filtration rate in rats. Am J Physiol. 1992;262:F361–6.
12. Schlondorff D. Roles of the mesangium in glomerular function. Kidney Int. 1996;49:1583–5.
13. Stockand JD, Sansom SC. Regulation of filtration rate by glomerular mesangial cells in health and diabetic renal disease. Am J Kidney Dis Off J Natl Kidney Found. 1997;29:971–81.
14. Wang X, Aukland K, Bostad L, Iversen BM. Autoregulation of total and zonal glomerular filtration rate in spontaneously hypertensive rats with mesangiolysis. Kidney Blood Press Res. 1997;20:11–7.
15. Almeida WS, Maciel TT, Di Marco GS, Casarini DE, Campos AH, Schor N. *Escherichia coli* lipopolysaccharide inhibits renin activity in human mesangial cells. Kidney Int. 2006;69:974–80.
16. Camussi G, Turello E, Tetta C, Bussolino F, Baglioni C. Tumor necrosis factor induces contraction of mesangial cells and alters their cytoskeletons. Kidney Int. 1990;38:795–802.
17. Maquigussa E, Arnoni CP, Cristovam PC, de Oliveira AS, Higa EM, Boim MA. *Escherichia coli* lipopolysaccharide impairs the calcium signaling pathway in mesangial cells: role of angiotensin II receptors. Exp Biol Med (Maywood). 2010;235:761–7.
18. Murray PT, Wylam ME, Umans JG. Endotoxin impairs agonist-induced calcium mobilization in rat mesangial cells. Am J Respir Crit Care Med. 1997;156:1846–54.
19. Ortiz-Arduan A, Danoff TM, Kalluri R, Gonzalez-Cuadrado S, Karp SL, Elkon K, et al. Regulation of Fas and Fas ligand expression in cultured murine renal cells and in the kidney during endotoxemia. Am J Physiol. 1996;271:F1193–201.
20. Sexton PM, Christopoulos G, Christopoulos A, Nylen ES, Snider RH Jr, Becker KL. Procalcitonin has bioactivity at calcitonin receptor family complexes: potential mediator implications in sepsis. Crit Care Med. 2008;36:1637–40.
21. Doi T, Vlassara H, Kirstein M, Yamada Y, Striker GE, Striker LJ. Receptor-specific increase in extracellular matrix production in mouse mesangial cells by advanced glycosylation end products is mediated via platelet-derived growth factor. Proc Natl Acad Sci USA. 1992;89:2873–7.
22. Iehara N, Takeoka H, Tsuji H, Imabayashi T, Foster DN, Strauch AR, et al. Differentiation of smooth muscle phenotypes in mouse mesangial cells. Kidney Int. 1996;49:1330–41.
23. Moriyama T, Fujibayashi M, Fujiwara Y, Kaneko T, Xia C, Imai E, et al. Angiotensin II stimulates interleukin-6 release from cultured mouse mesangial cells. J Am Soc Nephrol JASN. 1995;6:95–101.
24. Takeuchi Y, Yamauchi K, Nakamura J, Shigematsu S, Hashizume K. Angiotensin II regulates migration in mouse cultured mesangial cells: evidence for the presence of receptor subtype-specific regulation. J Endocrinol. 2006;191:361–7.
25. Rehli M, Luger K, Beier W, Falk W. Molecular cloning and expression of mouse procalcitonin. Biochem Biophys Res Commun. 1996;226:420–5.
26. Doi SQ, Jacot TA, Sellitti DF, Hirszel P, Hirata MH, Striker GE, et al. Growth hormone increases inducible nitric oxide synthase expression in mesangial cells. J Am Soc Nephrol JASN. 2000; 11:1419–25.
27. Lancaster JR Jr, Hibbs JB Jr. EPR demonstration of iron-nitrosyl complex formation by cytotoxic activated macrophages. Proc Natl Acad Sci USA. 1990;87:1223–7.
28. Beckman JS, Beckman TW, Chen J, Marshall PA, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA. 1990;87:1620–4.

29. de Jong HK, van der Poll T, Wiersinga WJ. The systemic pro-inflammatory response in sepsis. J Innate Immun. 2010;2:422–30.
30. Mathison JC, Wolfson E, Ulevitch RJ. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Investig. 1988;81:1925–37.
31. John E, Pais P, Furtado N, Chin A, Radhakrishnan J, Fornell L, et al. Early effects of lipopolysaccharide on cytokine release, hemodynamic and renal function in newborn piglets. Neonatology. 2008;93:106–12.
32. Dai C, Stolz DB, Kiss LP, Monga SP, Holzman LB, Liu Y. Wnt/beta-catenin signaling promotes podocyte dysfunction and albuminuria. J Am Soc Nephrol JASN. 2009;20:1997–2008.
33. He W, Dai C, Li Y, Zeng G, Monga SP, Liu Y. Wnt/beta-catenin signaling promotes renal interstitial fibrosis. J Am Soc Nephrol JASN. 2009;20:765–76.
34. He W, Kang YS, Dai C, Liu Y. Blockade of Wnt/beta-catenin signaling by paricalcitol ameliorates proteinuria and kidney injury. J Am Soc Nephrol JASN. 2011;22:90–103.
35. Surendran K, McCaul SP, Simon TC. A role for Wnt-4 in renal fibrosis. Am J Physiol Ren Physiol. 2002;282:F431–41.
36. Surendran K, Schiavi S, Hruska KA. Wnt-dependent beta-catenin signaling is activated after unilateral ureteral obstruction, and recombinant secreted frizzled-related protein 4 alters the progression of renal fibrosis. J Am Soc Nephrol JASN. 2005;16:2373–84.
37. von Toerne C, Schmidt C, Adams J, Kiss E, Bedke J, Porubsky S, et al. Wnt pathway regulation in chronic renal allograft damage. Am J Transplant Off J Am Soc Transplant Am Soc Transplant Surg. 2009;9:2223–39.
38. Kriz W, Elger M, Lemley K, Sakai T. Structure of the glomerular mesangium: a biomechanical interpretation. Kidney Int Suppl. 1990;30:S2–9.
39. Riser BL, Varani J, Cortes P, Yee J, Dame M, Sharba AK. Cyclic stretching of mesangial cells up-regulates intercellular adhesion molecule-1 and leukocyte adherence: a possible new mechanism for glomerulosclerosis. Am J Pathol. 2001;158:11–7.
40. Kikeri D, Pennell JP, Hwang KH, Jacob AI, Richman AV, Bourgoignie JJ. Endotoxemic acute renal failure in awake rats. Am J Physiol. 1986;250:F1098–106.
41. Lugon JR, Boim MA, Ramos OL, Ajzen H, Schor N. Renal function and glomerular hemodynamics in male endotoxemic rats. Kidney Int. 1989;36:570–5.
42. Chawla LS, Seneff MG, Nelson DR, Williams M, Levy H, Kimmel PL, et al. Elevated plasma concentrations of IL-6 and elevated APACHE II score predict acute kidney injury in patients with severe sepsis. Clin J Am Soc Nephrol CJASN. 2007;2:22–30.