ANTIOXIDANTS & REDOX SIGNALING
Volume 16, Number 5, 2012

DOI: 10.1089/ars.2011.4343

Do Cysteine Residues Regulate Transient Receptor Potential Canonical Type 6 Channel Protein Expression?

Florian Thilo,[1] Ying Liu,[2,3] Katharina Krueger,[1] Nora Förste,[1] Antje Wittstock,[1] Alexandra Scholze,[2] and Martin Tepel[1,2]

Abstract

The regulation of calcium influx through transient receptor potential canonical type 6 (TRPC6) channel is mandatory for the activity of human monocytes. We submit the first evidence that cysteine residues of homocysteine (HC) or acetylcysteine (ACC) affect TRPC6 expression in human monocytes. We observed that patients with chronic renal failure had significantly elevated HC levels and TRPC6 mRNA expression levels in monocytes compared with control subjects. We further observed that administration of HC or ACC significantly increased TRPC6 channel protein expression compared with control conditions. We, therefore, hypothesize that cysteine residues increase TRPC6 channel protein expression in humans. *Antioxid. Redox Signal*. 16, 452–457.

Introduction

Transient receptor potential canonical type 6 (TRPC6) channel induced cation influx in activated human monocytes plays an important role in the pathogenesis of atherosclerosis. The latter has been considered an inflammatory disease with accumulation of monocytes within the artery wall. Monocytes are transitional cells, with a short half life due to rapid differentiation into macrophages that are rapidly recruited to sites of inflammation. Monocyte activation, adhesion to the endothelium, and transmigration into the subendothelial space are critical events in the early pathogenesis of atherosclerosis.

The regulation of calcium influx through TRPC6 channels is mandatory for controlling the activity of human monocytes. There are evidences that this regulation of calcium influx through TRPC6 channels may be controlled by the redox state. Wuensch *et al.* showed that high glucose levels increased both reactive oxygen species and TRPC6 expression (8). Furthermore, several TRPC channels are activated by oxidants, which induce sodium and calcium entry into cells through mechanisms that are dependent on phospholipase C (4).

It is well known that homocysteine (HC) is significantly increased in patients with chronic renal failure (CRF), thereby affecting atherosclerotic diseases (6). Currently, it is unknown whether the increased atherosclerotic burden in patients with CRF may be aggravated by the effects of HC on TRPC6 channel expression in monocytes. HC as well as acetylcysteine

Innovation

Activation of monocytes, which plays a critical role in the development of atherogenesis, is dependent on calcium influx through transient receptor potential canonical type 6 channel (TRPC6) channels. Regulation of calcium influx through TRPC6 channels may be controlled by the redox state, as Wuensch *et al.* have recently shown that high glucose levels increase both reactive oxygen species and TRPC6 expression. Monocyte function is altered by acetylcysteine (ACC) as well as by homocysteine (HC), both of which contain cysteine residues. The effects of ACC are dependent on the redox milieu, that is, while ACC is mainly known as an antioxidant, it can also exert prooxidative effects by autooxidation of ACC. This study describes observations of the effects of ACC and HC on TRPC6 expression in human monocytes *in vivo* and *in vitro* and concludes with the hypothesis that the noticed increase of TRPC6 expression by ACC and HC might be caused by the cysteine residues in HC and ACC, influencing the intracellular redox state. Since experimental evidence of differential expression of TRPC6 and other TRPC channels in various cardiovascular disease settings is mounting, insight into the role that changes in redox state play on the expression of TRPC6 and other TRPC channels is highly critical for correct interpretation of expression profiles.

[1]Department of Medicine, Division of Nephrology, Charité Campus Benjamin Franklin, Berlin, Germany.
[2]Odense University Hospital, Department of Nephrology, and University of Southern Denmark, Institute of Molecular Medicine, Cardiovascular and Renal Research, Institute of Clinical Research, Odense, Denmark.
[3]Department of Urology, Tenth People's Hospital Tongji University, Shanghai, People's Republic of China.

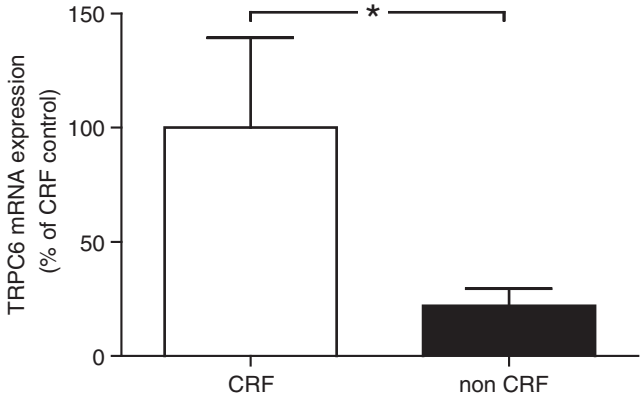

FIG. 1. TRPC6 mRNA expression was significantly higher in monocytes from CRF patients compared to non-CRF control subjects. Bar graphs showing transient receptor potential canonical type 6 channel (TRPC6) mRNA expression (normalized ratio) in monocytes from 17 patients with chronic renal failure (CRF) and 19 control patients without CRF (non CRF). *$p < 0.05$.

(ACC) contain cysteine residues that influence the intracellular redox state. Furthermore, HC and ACC are known to affect monocyte function (3, 7). In the present study, we investigated whether HC or ACC may affect TRPC6 expression in human monocytes, both in control subjects and in patients with CRF.

Results, Discussion, and Future Directions

To evaluate whether increased cysteine residues are associated with increased TRPC6 transcripts, we compared TRPC6 mRNA in monocytes from 17 patients with CRF (6 women, 11 men; mean age 38 ± 3 years; systolic blood pressure 136 ± 7 mmHg; diastolic blood pressure 71 ± 4 mmHg; serum sodium 135 ± 1 m*M*, serum potassium 4.7 ± 0.1 m*M*, hemoglobin 10.3 ± 0.4 g/dl, serum creatinine 6.20 ± 0.84 mg/dl) and 19 non-CRF control subjects (9 women, 10 men; mean age 68 ± 3 years; systolic blood pressure 138 ± 5 mmHg; diastolic blood pressure 83 ± 2 mmHg; serum sodium 136 ± 1 m*M*, serum potassium 4.2 ± 0.1 m*M*, hemoglobin 12.9 ± 0.4 g/dl, serum creatinine 0.98 ± 0.06 mg/dl). CRF patients had significantly elevated HC levels compared with non-CRF control subjects (23.8 ± 3.6 μM *vs.* 11.3 ± 1.0 μM; $p < 0.01$). TRPC6 mRNA expression was significantly higher in monocytes from CRF patients compared with non-CRF control subjects (normalized ratio, 100 ± 39 *vs.* 22 ± 7 arbitrary units, $p < 0.01$) (Fig. 1).

Now, the effects of substances containing cysteine residues on TRPC6 channel protein expression were analyzed *in vitro*. In monocytes from CRF patients, the administration of HC (100 μM) or ACC (500 μg/ml) significantly increased TRPC6 channel protein expression compared with control conditions (normalized ratio, 195 ± 2 *vs.* 100 ± 3 arbitrary units for HC; normalized ratio, 117 ± 3 *vs.* 100 ± 3 arbitrary units for ACC; each $p < 0.05$; $n = 8$). In monocytes from non-CRF control subjects, a similar effect could be observed (normalized ratio, 136 ± 1 *vs.* 100 ± 7 arbitrary units for HC; normalized ratio, 125 ± 9 *vs.* 100 ± 7 arbitrary units for ACC; $p < 0.05$; each $n = 8$) (Fig. 2). As shown in Figure 3 the effect of ACC on TRPC6 protein expression was time- and concentration-dependent.

HC altered TRPC6 protein in the same manner as did ACC. The effects of HC and ACC were even more pronounced in monocytes from CRF patients. A possible explanation for

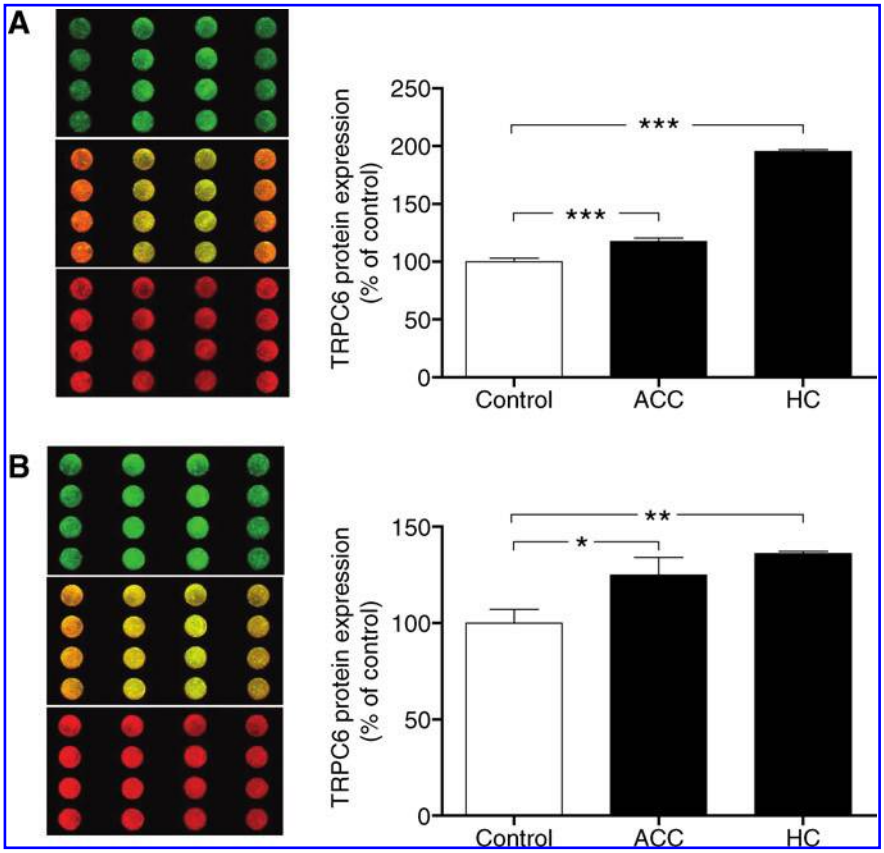

FIG. 2. Administration of HC or ACC significantly increased TRPC6 channel protein expression in monocytes. Quantitative in-cell Western assay of TRPC6 channel protein expression in monocytes of patients with CRF and control patients without CRF, treated with acetylcysteine (ACC) (500 μg/ml) or homocysteine (HC) (100 μM). **(A)** Summary data of TRPC6 channel protein expression in monocytes of patients with CRF, treated with ACC (500 μg/ml) or HC (100 μM). Data are mean ± standard error of the mean (SEM) from at least four independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control conditions (TRPC6 protein [*green*], glyceraldehyde-3-phosphate-dehydrogenase [GAPDH] protein [*red*] for loading control, and overlay [*orange-yellow*]). **(B)** Summary data obtained in monocytes from control patients without CRF. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

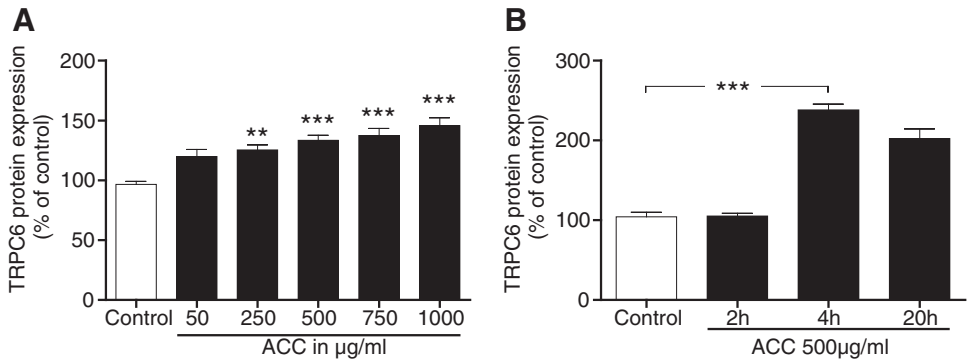

FIG. 3. The effect of ACC on TRPC6 channel protein expression in monocytes was time- and concentration-dependent. (A) TRPC6 protein expression in relation to various incubation time periods. Data are mean ± SEM from at least four independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control conditions. **(B)** TRPC6 protein expression in relation to various ACC concentrations.

these experimental results is that cysteine residues affect posttranslational TRPC6 processing. Different changes of redox activities have been described after administration of HC or ACC depending on the local redox milieu (1). The redox state of the cellular milieu, for instance, depends on the level of serum present in the cell culture.

To evaluate whether the HC or ACC-induced TRPC6 protein expression was able to affect intracellular phosphorylation and signal transduction, we measured extracellular-signal-regulated kinase (ERK) and phosphorylated ERK protein expression in monocytes after incubating with ACC (5) for 4 h at 37°C by in-cell western assay. As shown in Figure 4, ACC in a dose of 500 μg/ml significantly increased phosphorylated ERK (pERK) protein expression compared with control conditions (normalized ratio, 226 ± 17 *vs.* 100 ± 12; $p < 0.05$; $n = 6$). This is in line with studies reporting an activation of ERK under the influence of ACC (9). The increased phosphorylation of ERK, therefore, supports oxidative stress (1). This effect was abolished in the presence of TRPC blockers, that is, gadolinium or 2-aminoethoxydiphenyl borate (69 ± 3 arbitrary units or 85 ± 3 arbitrary units, respectively). TRPC6, therefore, seems to be involved in the signaling process by which HC and ACC influence phosphorylation and intracellular signaling.

Since ACC increases TRPC6 protein expression we measured whether intracellular calcium might be elevated after administration of ACC in patients with end-stage renal failure, who were routinely dialyzed three times weekly for 4 h.

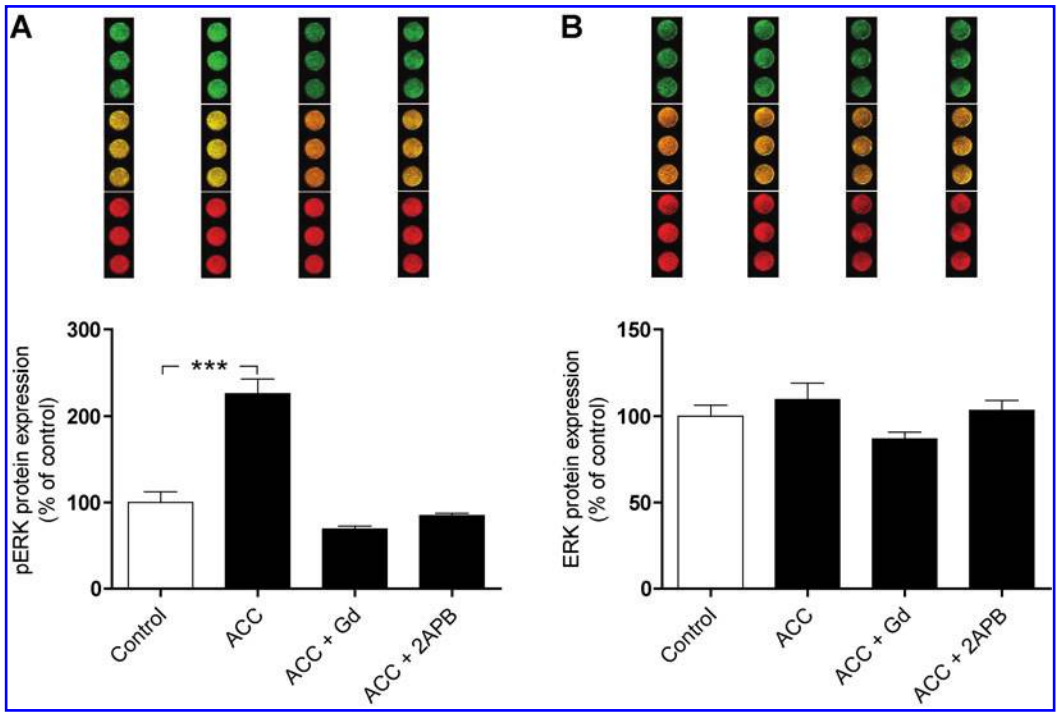

FIG. 4. ACC significantly increased pERK protein expression compared to control conditions. Quantitative in-cell Western assay of phosphorylated extracellular-signal-regulated kinase (pERK) and ERK protein expression in monocytes treated with ACC (500 μg/ml) in the absence (control) and presence of gadolinium (Gd; 50 μM), 2-aminoethoxydiphenyl borate (2-APB; 100 μM). Data are mean ± SEM from at least four independent experiments. $^{***}p < 0.001$ compared to control conditions. **(A)** Quantitative in-cell Western assay of pERK protein expression in monocytes (pERK protein [*green*], GAPDH protein [*red*] for loading control, and overlay [*orange-yellow*]). **(B)** Quantitative in-cell Western assay of ERK protein expression in monocytes (ERK [*green*], GAPDH protein [*red*] for loading control, and overlay [*orange-yellow*]). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Table 1. Clinical and Biochemical Characteristics of 20 Patients with End-Stage Renal Failure

	In absence of acetylcysteine		*In presence of acetylcysteine*	
Characteristics	*Before hemodialysis*	*After hemodialysis*	*Before hemodialysis*	*After hemodialysis*
Age (years)	69 ± 2		69 ± 2	
Female (%)	20		20	
Body weight (kg)	69 ± 3		69 ± 3	
Body mass index (kg/m^2)	21.2 ± 1.7		21.7 ± 1.7	
Ultrafiltration/weight (100 × L/kg)	3.9 ± 0.3		3.5 ± 0.2	
Systolic blood pressure (mmHg)	137 ± 5	136 ± 6	138 ± 6	137 ± 6
Diastolic blood pressure (mmHg)	68 ± 3	68 ± 3	71 ± 4	67 ± 2
Pulse pressure (mmHg)	69 ± 4	68 ± 5	67 ± 4	69 ± 4
Hematocrit (%)	29 ± 0	30 ± 0	31 ± 0	30 ± 0
Leukocytes (/nl)	6.9 ± 0.7	7.5 ± 0.8	6.7 ± 0.6	6.8 ± 0.7
Hemoglobin (g/dl)	9.6 ± 0.4	10.1 ± 0.4	10.2 ± 0.4	10.2 ± 0.4
Platelets (/nl)	195 ± 22	193 ± 20	202 ± 26	210 ± 24
Serum creatinine (μM)	605 ± 46	255 ± 29	582 ± 41	253 ± 22
Blood urea nitrogen (m*M*)	21 ± 2	6 ± 1	20 ± 2	12 ± 5
Total protein (g/L)	63 ± 1	67 ± 2	64 ± 1	67 ± 2
Serum sodium (m*M*)	136 ± 1	137 ± 1	136 ± 1	140 ± 1
Serum potassium (m*M*)	4.8 ± 0.1	3.9 ± 0.1	5.0 ± 0.1	4.0 ± 0.1
Serum calcium (m*M*)	2.4 ± 0.0	2.7 ± 0.0	2.4 ± 0.0	2.7 ± 0.0
Serum phosphate (m*M*)	1.5 ± 0.1	0.9 ± 0.1	1.5 ± 0.1	0.8 ± 0.1
Plasma homocysteine (m*M*)	20.2 ± 2.0	13.4 ± 1.7	21.7 ± 2.3	2.7 ± 0.3

Continuous data are shown as mean ± standard error of the mean.

Hemodialysis was performed in the absence (control) and presence of intravenous ACC. The clinical and biochemical characteristics of the patients are shown in Table 1. Similar ultrafiltration ratios were observed (control 3.9% ± 0.3%, ACC 3.5% ± 0.2%; $p = 0.29$), predialysis hematocrit (control 29% ± 0%, ACC 31 ± 0; $p = 0.25$), predialysis serum protein (control 63 ± 1 g/L, ACC 64 ± 1; $p = 0.14$), and predialysis serum creatinine concentrations (control 605 ± 46 μM, ACC 582 ± 41 μM; $p = 0.66$). During hemodialysis treatment, we detected a significant decrease ($p < 0.001$) of serum creatinine (control, 605 ± 46 to 255 ± 29 μM; ACC, 582 ± 41 to 253 ± 22 μM), blood urea nitrogen (control, 21 ± 2 to 6 ± 1 m*M*; ACC, 20 ± 2 to 12 ± 5 m*M*), serum potassium (control, 4.8 ± 0.1 to 3.1 ± 0.1 m*M*; ACC, 5.0 ± 0.1 to 4.0 ± 0.1 m*M*), and serum phosphate (control, 1.5 ± 0.1 to 0.9 ± 0.1 m*M*; ACC, 1.5 ± 0.1 to 0.8 ± 0.1 m*M*). The transplasmamembrane calcium influx was significantly higher at the end of a hemodialysis session in the presence of ACC (Fig. 5A). Furthermore, chlorotetracycline-labeling of intracellular calcium stores revealed that the calcium concentration in intracellular stores was significantly increased after a hemodialysis session in the presence of ACC (Fig. 5B). Similarly, the thapsigargin-induced calcium release from intracellular stores was significantly higher after a hemodialysis session in the presence of ACC (Fig. 5C). These data indicate that increased TRPC6 protein expression after administration of ACC significantly increases transplasmamembrane calcium influx and intracellular stored calcium.

In conclusion, the present study suggests that cysteine residues increase TRPC6 channel protein expression in humans. The augmented TRPC6 channel protein expression was accompanied by increased phosphorylated ERK and increased transplasmamembrane calcium influx and intracellular stored calcium.

Future studies on TRPC6 and other TRPC channels in various clinical and experimental settings are, therefore, warranted in which differential expression patterns are interpreted in the light of changes in redox state.

Notes

Preparation of cells

Human monocytes were obtained from heparinized blood. All subjects gave written informed consent, and the study was approved by the local ethics committee. Monocytes were separated using superparamagnetic polystyrene beads coated with a primary monoclonal antibody specific for the CD14 membrane antigen expressed on human monocytes (Invitrogen, Groningen, Germany) and resuspended in Hanks balanced solution containing (in m*M*) NaCl 136, KCl 5.40, $CaCl_2$ 1, KH_2PO_4 0.44, Na_2HPO_4 0.34, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid 10, and pH 7.4. Monocytes were counted in Neubauer's chamber and adjusted in each experiment.

RNA isolation and reverse transcription

Total RNA was isolated from monocytes using the RNeasy mini kit including RNase-free DNase set (Qiagen, Hilden, Germany). Using the Transcriptor first-strand cDNA synthesis kit (Roche Diagnostics, Mannheim, Germany), cDNA was synthesized from total RNA using oligo dT (12–18) and 5 U avian myeloblastosis virus (AMV) reverse transcriptase at 50°C for 60 min, followed by heating to 85°C for 5 min.

Quantitative real-time reverse transcriptase–polymerase chain reactions

Quantitative real-time reverse transcriptase–polymerase chain reactions (qRT-PCR) for TRPC6 and glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) were performed using a LightCycler-FastStart DNA Master SYBR Green I Kit (Roche Diagnostics). The primers were as follows:

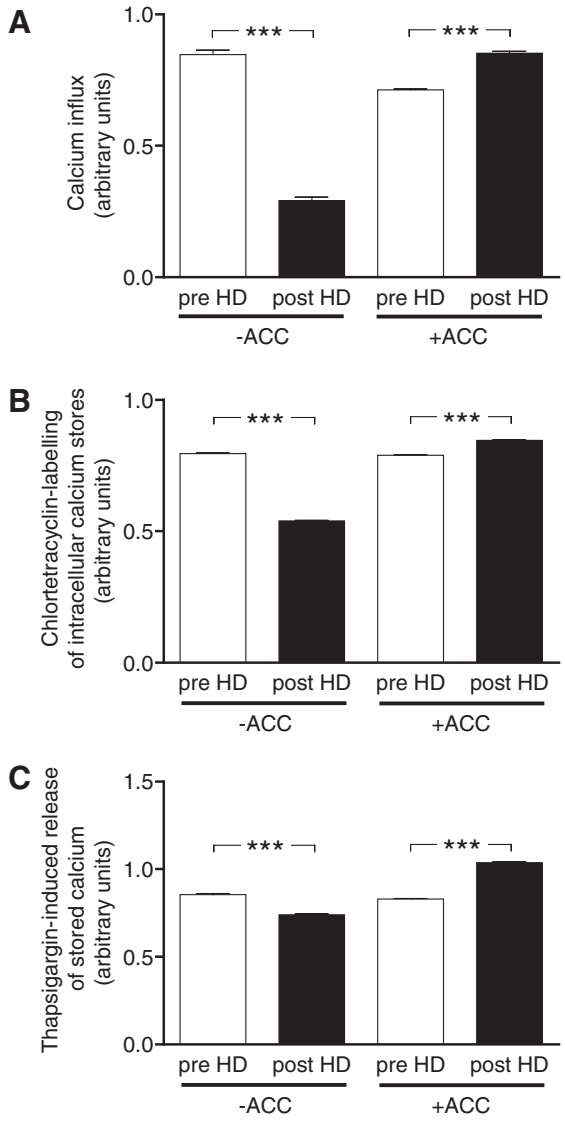

FIG. 5. Increased TRPC6 protein expression after administration of ACC significantly increased transplasmamembrane calcium influx and intracellular stored calcium. Measurements were performed at the start (*open bars*) and at the end (*filled bars*) of a hemodialysis session in absence (−ACC) and presence (+ACC) of intravenous ACC in 20 patients with end-stage renal failure. Data are mean ± SEM. ***$p < 0.001$. **(A)** Transplasmamembrane calcium influx. **(B)** Chlorotetracycline-labeling of intracellular calcium stores. **(C)** Thapsigargin-induced release of stored calcium in mononuclear leukocytes.

TRPC6 (NM 004621),
forward, 5′ GCCAATGAGCATCTGGAAAT 3′,
reverse, 5′ TGGAGTCACATCATGGGAGA 3′;
GAPDH (NM 002046),
forward, 5′ AACTGCTTAGCACCCCTGGC 3′,
reverse, 5′ ATGACCTTGCCCACAGCCTT 3′.

The expected and observed sizes of the PCR products were 243 bp for TRPC6 and 200 bp for GAPDH.

LightCycler-Fast Start DNA SYBR Green I mix (Roche Diagnostics) and 500 n*M* of each primer were used in a final volume of 20 μL. The reaction was initiated at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing for 10 s at 57°C (TRPC6) or at 54°C (GAPDH), and extension at 72°C for 15 s. Melting curve analysis was performed from 65°C to 95°C with a heating rate of 0.1°C/s. Data were recorded on a LightCycler 2.0 Instrument using LightCycler Software Version 4.0 (Roche Diagnostics).

The relative quantification method was used whereby the change in expression of the target gene (*TRPC6*) relative to the housekeeping gene (*GAPDH*) was calculated.

Immunofluorescence assay

For the identification of TRPC6 channel proteins, quantitative in-cell Western assays of human monocytes were performed using the Odyssey infrared imaging system (Licor Biosciences, Bad Homburg, Germany). Human monocytes in 96-well plates were incubated with rabbit anti-human TRPC6-antibodies (1: 1000; Alomone Labs, Jerusalem, Israel), ERK, and pERK (1: 1000; Santa Cruz Biotechnology, Santa Cruz, CA), as well as GAPDH (1: 1000; Santa Cruz Biotechnology) or CD11a (1: 1000; Serotec, Duesseldorf, Germany) for internal reference for 2 h, washed, and incubated with IRDye800-infrared fluorescent dye-conjugated sheep anti-rabbit antibodies (1: 1000; Biomol, Hamburg, Germany) overnight at 4°C. Imaging was performed at 810 nm emission with an excitation wavelength of 780 nm. Control experiments were performed with omission of primary or secondary antibodies.

Conventional immunoblotting

For conventional immunoblotting, monocytes were washed with phosphate-buffered saline, transferred to ice-cold high-salt lysis buffer (containing 25 m*M* Tris–HCl, pH 8; 1 *M* NaCl, 200 m*M* ethylenediamine tetraacetic acid, 1 *M* ß-mercaptoethanol, 1 *M* sodium fluoride, complete mini protease inhibitor cocktail (Roche Diagnostics), repeatedly aspirated through a syringe needle, and heated to 90°C for 3 min. Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis at 150 V for 90 min, and transferred to Hybond-ECL nitrocellulose membranes (NEN Life Science Products, Boston, MA). Membranes were blocked with Odyssey blocking buffer (Licor Biosciences) for 24 h at 4°C. Membranes were incubated with rabbit anti-human TRPC6 antibodies (Alomone Labs) at 1: 500 solution containing 0.1% Tween 20 for 60 min and washedthrice; they were then incubated with IRDye800CW-infrared fluorescent dye-conjugated sheep anti-rabbit antibodies (Biomol) at 1: 1000 solution containing 0.1% Tween 20 for 60 min, and washed thrice. Imaging was performed at 810 nm emission with an excitation wavelength of 780 nm.

Measurements of cytosolic calcium using fluorescent dye technique

For ratiometric imaging, experiments monocytes were loaded with 2 μM of the calcium-sensitive, cell-permeable, intracellular fluorescence dye 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2′-amino-5′-methyl-phenoxy)-ethane-*N*,*N*,*N*′*N*′-tetra acetic acid penta acetoxymethyl ester (fura-2/AM; Merck Biosciences, Darmstadt, Germany) at room temperature for 40 min and washed to remove extraneous dye. Fluorescence measurements were performed in a temperature-controlled 96-well-fluorescent plate reader at 37°C (Fluoroskan Ascent Fluorometer; Thermo LabSystems Oy, Helsinki, Finland) at 510 nm emission with excitation wavelengths of 340 nm and 380 nm. Baseline fluorescence

was measured for 10 min, and stable fluorescence readings were obtained throughout.

Thapsigargin (Sigma Aldrich, Steinheim, Germany; final concentration 1 μM), a specific inhibitor of sarcoendoplasmic reticulum Ca^{2+}-ATPase, was used to deplete calcium stores. Calcium content of intracellular stores was also measured by chlorotetracycline-labeling (Sigma Aldrich; final concentration 10 μM) using 530 nm emission with an excitation wavelength of 380 nm. Fluorescence showed an instantaneous increase followed by a slow increase of fluorescence, the latter being due to fluorescence arising from intracellular calcium stores.

Patients

For mRNA and protein analysis, 17 patients with CRF and 19 control subjects without CRF (non-CRF controls) were enrolled. The local ethics committee approved the study protocol, and all patients gave written informed consent. To investigate the effects of ACC *in vivo*, patients with end-stage renal failure were enrolled in this study. In a prospective, cross-over, randomized design, each patient received ACC (5 g in 5% glucose solution for 4 h) during a single hemodialysis session and 5% glucose solution alone for placebo control during another hemodialysis session. The ACC dose was in a range that has been reported to reduce plasma HC concentration efficiently (6). All patients were routinely dialyzed thrice weekly for 4 h using a biocompatible polysulfone membrane (F8; Fresenius Medical Care) with no dialyzer reuse. The dialyze solutions used were bicarbonate based. Kt/V values (the amount of plasma cleared of urea divided by the urea distribution volume) were measured according to Daugirdas' formula (2) with $Kt/V = (-1) * \log(\text{ratio} - (0.03)) + ((4 - (3.5 * \text{ratio})) * (\text{ultrafiltrate volume/weight}))$, with ratio = post blood urea nitrogen (BUN)/pre BUN. The local ethic committee approved the study protocol, and all patients gave written informed consent.

Statistical analysis

All values are reported as mean ± standard error of the mean. Comparisons between groups were analyzed using non-parametric Mann–Whitney test (GraphPad Prism 5.0; LaJolla, CA). Data from multiple groups were analyzed using the non-parametric Kruskal–Wallis test and Dunn's multiple comparison *post hoc* test. Two-sided *p*-values below 0.05 were considered as indicating statistical significance.

Acknowledgments

The study was supported by the Else-Kröner-Fresenius-Stiftung, Germany.

Author Disclosure Statement

No competing financial interests exist.

References

1. Chan ED, Riches DW, and White CW. Redox paradox: effect of N-acetylcysteine and serum on oxidation reduction-sensitive mitogen-activated protein kinase signaling pathways. *Am J Respir Cell Mol Biol* 24: 627–632, 2001.
2. Daugirdas JT. Second generation logarithmic estimates of single-pool variable volume Kt/V: an analysis of error. *J Am Soc Nephrol* 4: 1205–1213, 1993
3. Jensen T, Kharazmi A, Schiøtz PO, Nielsen H, Stenvang Pedersen S, Stafanger G, Koch C, and Høiby N. Effect of oral N-acetylcysteine administration on human blood neutrophil and monocyte function. *APMIS* 96: 62–67, 1988.
4. Miller BA. The role of TRP channels in oxidative stress-induced cell death. *J Membr Biol* 209: 31–41, 2006.
5. Roux PP and Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68: 320–344, 2004.
6. Scholze A, Rinder C, Beige J, Riezler R, Zidek W, and Tepel M. Acetylcysteine reduces plasma homocysteine concentration and improves pulse pressure and endothelial function in patients with end-stage renal failure. *Circulation* 109: 369–374, 2004.
7. Splaver A, Lamas GA, and Hennekens CH. Homocysteine and cardiovascular disease: biological mechanisms, observational epidemiology, and the need for randomized trials. *Am Heart J* 148: 34–40, 2004.
8. Wuensch T, Thilo F, Krueger K, Scholze A, Ristow M, and Tepel M. High glucose-induced oxidative stress increases transient receptor potential channel expression in human monocytes. *Diabetes* 59: 844–849, 2010.
9. Yan CY and Greene LA. Prevention of PC12 cell death by N-acetylcysteine requires activation of the Ras pathway. *J Neurosci* 18: 4042–4049, 1998.

Address correspondence to:
Dr. Florian Thilo
Department of Nephrology
Charité Campus Benjamin Franklin
Thielallee 71
14195 Berlin
Germany

E-mail: florian.thilo@charite.de

Date of first submission to ARS Central, October 16, 2011; date of acceptance, October 17, 2011.

Abbreviations Used

2-APB = 2-aminoethoxydiphenyl borate
ACC = acetylcysteine
BUN = blood urea nitrogen
CRF = chronic renal failure
ERK = extracellular-signal-regulated kinase
GAPDH = glyceraldehyde-3-phosphate-dehydrogenase
Gd = gadolinium
HC = homocysteine
PCR = polymerase chain reaction
pERK = phosphorylated ERK
TRPC6 = transient receptor potential canonical type 6 channel