CKD Uraemic Environment does not Influence Adiponectin Secretion- A “Bed-To-Bench” Approach

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Abstract

Adiponectin (ADPN) is an adipocyte-derived protein carrying anti-atherogenic properties in the general population. Controversially, ADPN serum concentrations are positively associated with the magnitude of chronic kidney disease (CKD), which is the strongest cardiovascular risk factor per se. This piece of reverse epidemiology raises the question, if ADPN is stimulated by uremia itself or is simply a bystander of CKD. To enlighten this question on the cellular level, we performed co-stimulatory experiments in human adipocytes using insulin- an established ADPN stimulating agent- and compound uremic haemofiltrate from patients with CKD stage 5. Haemofiltrate was employed as a model fluid because the more comprehensive approach of using uremic serum was methodologically impossible due to the ADPN content of serum.

Differentiated adipocytes were treated with compound uraemic haemofiltrate from patients with CKD stage 5 or PBS as control. Surprisingly, we did not detect any additional stimulatory effect of increasing haemofiltrate concentrations on adiponectin secretion of human adipocytes after 24 and 48 hours of incubation (p=0.67 or 0.07 resp.).

Therefore, this in vitro experiment preliminary precludes a pathophysiological effect of water-soluble uremic compounds on ADPN cellular secretion. Further experiments with varying amounts of insulin and other ADPN stimulators are needed to confirm such preclusion.

Introduction

Adiponectin (ADPN) is an adipocyte-derived protein [1] with potential antiatherogenic properties and exclusively secreted by adipocytes. In chronic kidney disease (CKD), ADPN serum levels are increased [2-7] for unknown reasons although accumulation due to abolished clearance [8-10] or modified metabolic pathways [4] has been hypothesized but not finally proved. The ADPN increase in CKD is of certain biological interest, because in the general population ADPN serum levels are inversely associated with cardiovascular risk [11-15]. The underlying vascular-protective effects are mounted on several mechanisms like insulin sensitizing [16-19], maintaining endovascular homeostasis [20] and anti-inflammatory properties [21,22]. In opposite, patients with chronic kidney disease (CKD) are subjected to an increased cardiovascular risk [23,24] in general and by even higher extent, if they exhibit higher ADPN serum values [3,6,25,26]. Therefore, the finding of increased ADPN in CKD and increased mortality risk along with higher ADPN can be regarded as a paradox or a further finding of reverse epidemiology [5]. It must be challenged, whether in CKD ADPN plays the same protective role as it is supposed to do in the general population.

Osteoclast activation in CKD may be regarded as one of the candidate mechanisms to be differentially effective in CKD vs. normal environment [27]. Because osteoclasts are being activated via NFκB [28] and the Receptor Activator of NFκB Ligand (RANKL) [29], this pathway could be seen as a hypothetical interplay with ADPN regulation. ADPN was shown to stimulate RANKL in vitro and inhibited osteoprotegrin (OPG) in osteoblasts. In opposite, ADPN was found to be positively associated with OPG [30], which is indeed a decoy receptor for NFκB and therefore protects endothelial integrity [31,32]. Hypothetically, the molecular role of ADPN in CKD could be seen inversely to it’s role in other populations, since it’s induction might activate osteoclasts and calcium-phosphate liberation from bones becoming osteopenic. With such regard, the impact of ADPN could be a function of renal disease and the particular milieu in CKD.
As a step to clarify the underlying mechanism we aimed to decipher whether ADPN secretion by adipocytes might be influenced by uremia itself, initially by deployment of a comprehensive variety of uremic compounds as stimulating agent for ADPN secretion of adipocytes. Because serum of CKD patients contains such comprehensive assortment, but also ADPN in a large quantity, it is technically difficult or not possible to separate detection results between serum ADPN and ADPN secreted by adipocytes after stimulation. The present study examined insulin-stimulated nearly mature adipocytes incubated with uremic, but ADPN-free haemofiltrate from end-stage, dialysis-necessitating CKD patients to analyse ADPN secretion under uremic conditions.

Methods

Cell culture

To test ADPN secretion, preadipocytes isolated from a patient with Simpson-Golabi-Behmel syndrome (SGBS) were studied. These immortal cells can easily be differentiated into nearly mature adipocytes by insulin and are specifically useful to study adipocyte differentiation, metabolism, and secretory properties.

Cells were seeded in culture flasks by a density of 4000/cm² and cultivated in Dulbecco’s Modified Eagle’s Medium (DMEM) (Invitrogen, Karlsruhe, Germany). When cell confluence reached 80%, cells were washed threefold with phosphate-buffered saline (PBS, Invitrogen) and insulin-induced 3 days with DMEM enriched by 20µM insulin, 10% fetal calf serum, 30mm Biotin (Sigma-Aldrich, Seelze, Germany), 150 mm Panthothenat (Sigma-Aldrich, Seelze, Germany), 1000U/ml Penicillin (Sigma-Aldrich, Seelze, Germany) and 100mg/ml Streptomycin (Sigma-Aldrich, Seelze, Germany), in order to induce adipogenic differentiation.

After 9 days, differentiation medium (insulin-containing) was replaced by the investigational (insulin-free) culture medium. That medium consisted of DMEM with varying amounts of pooled uremic haemofiltrate (HF) or PBS. To adjust for changes in nutrient concentrations in dilution series, PBS or uremic solution were added at similar volumes yielding constant nutrient concentrations in dilution series, PBS or uremic concentration was conducted twice with triplicate ADPN measurement at each time-point. Experiment B used variable uremic haemofiltrate concentrations of 0, 10, 20, 30, 40 and 50% (with the replacing amount of PBS, resp.) at time points 24 and 48 hours with ADPN measurement in triplicate. Every uremic concentration was conducted twice with triplicate ADPN measurement. Total ADPN was measured by sheep alkaline-phosphatase based antibody ELISA assay (Biovendor, Heidelberg, Germany, Cat.# RSCHHMWADDN096R). The assay was calibrated according the user’s manual by means of a pretested calibration curve. Each data-point displays the mean of 3 measurements and standard deviation.

Statistical analysis

For comparison of correlations between uremic concentration, incubation time and ADPN concentration, linear regression and ANOVA was used. The mean ADPN change per % change of HF proportion is given as 95% confidence interval. For comparison of 24h vs. 48h time points and uremic augmentation vs. PBS, paired t-test was used.

Results

Figure 1: ADPN secretion of SGBS adipocytes at different time points with 30% haemofiltrate supplementation or PBS supplementation as control. ADPN measurements in triplicate.

Supernatant total ADPN concentration and time of incubation were highly correlated both with (Pearson’s R 0.92; p=0.003).
and without (Pearson’s R 0.99; p<0.0001) 30% HF co-incubation (Figure 1). At time points 24 and 48 hours, the supernatant ADPN concentration was higher in uremic solutions (p<0.001, p=0.007) compared to PBS while the incremental increase of ADPN with augmented uremic environment did not reach correlation with the incremental increase of ADPN over time (p=0.1, Figure 1).

Supernatant total ADPN concentration and concentration of uremic co-incubation fluid were not correlated at 24 hours (Pearson’s R 0.14; p=0.67) and 48 hours (Pearson’s R-0.59; p<0.067) (Figure 2).

ADPN levels in both non-uremic and serial augmented uremic environment were about 10 times higher (95%CI Δ 162...195; p<0.0001) after an incubation period of 48 hours compared to 24 hours (p<0.0001).

Discussion
By applying a uremic environment of small water soluble molecules on secreting adipocytes in an artificial setting, overt and continuing ADPN stimulation was not notable on the background of initial one-time insulin stimulation. The 1.8fold ADPN increase responding to 30% uremic concentration was only present at time-points 24 and 48 hours, was not disproportionate to the ADPN time dependency and could not be replicated with varying concentrations of uremic environment. We applied an assay based on SGBS cells comparable to other in vitro studies with a similar technical approach. The tremendous time course of ADPN secretion may indicate that peak ADPN secretion was not reached at the 24-hours-time-point and our experiments were done in a linear phase of the ADPN time-secretion relationship. Such reasoning points to the assumption, that uremic environment was present during a non-plateau and secreting phase of ADPN production but did not carry major stimulatory effects exceeding insulin effects. These results can be interpreted as a preliminary preclusion of an in-vitro stimulatory effects exceeding insulin effects. These results replicated with varying concentrations of uremic environment. The tremendous time course of ADPN secretion may indicate that peak ADPN secretion was not reached at the 24-hours-time-point and our experiments were done in a linear phase of the ADPN time-secretion relationship. Such reasoning points to the assumption, that uremic environment was present during a non-plateau and secreting phase of ADPN production but did not carry major stimulatory effects exceeding insulin effects. These results can be interpreted as a preliminary preclusion of an in-vitro stimulatory effects exceeding insulin effects. These results replicated with varying concentrations of uremic environment.

In vitro studies like the present one carry shortcomings when comparisons to clinical situations are drawn. First, by using concentrated haemofiltrate from the first period of conventional dialysis, we were able to draw a mixture of small water-soluble, but not protein-bound and larger molecules because these molecules do pass the dialysis filter membrane by only a minor proportion [34]. Methodological, we tested total ADPN, although distinct biological effects of HMW isoforms have been suggested [35]. However, there is available evidence that total and HMW ADPN are correlated and total ADPN assays have been applied as satisfying surrogates [14]. Of course, biological cofactors like lipid metabolism, inflammation and bone metabolism could not have been simulated. The present results were mounted on the background of initial insulin stimulation to accomplish adipogenic differentiation. By such procedure we aimed to reach comparison with known physiological mechanism (insulin dependency) with the hypothesized uraemia dependency. Because this was not the case, we may conclude that uraemia at least does not play a role as insulinemia does. We cannot draw conclusions regarding smaller increments, e.g. in terms of not insulin-dependent systems.

Therefore our initial investigational findings do only add first reasoning that adipocyte ADPN secretion is not enhanced overtly by uraemia. Further steps, using modified insulin and other co-stimulating compounds as well as protein-bound compounds seem to be meaningful to widen the perception of cellular ADPN secretion in frame of CKD.

References
1. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 270(45): 26746-26749.
2. Bakkaloglu SA, Buyan N, Funahashi T, Pasagolu H, Elhan AH, et al. (2005) Adiponectin levels and atherosclerotic risk factors in pediatric chronic peritoneal dialysis patients. Perit Dial Int 25(4): 357-361.
3. Bakkaloglu SA, Soykemezoglu O, Buyan N, Oktar SO, Funahashi T, et al. (2006) Adiponectin levels and atherosclerotic risk factors in pediatric renal transplant recipients. Pediatr Transplant 10(2): 187-192.
4. Bakkaloglu SA, Soykemezoglu O, Buyan N, Funahashi T, Elhan AH, et al. (2005) High serum adiponectin levels during steroid-responsive nephrotic syndrome relapse. Pediatr Nephrol 20(4): 474-477.
5. Beige J, Heimann K, Stumvoll M, Korner A, Kronsbein J (2009) Paradoxical role for adiponectin in chronic renal disease? An example of reverse epidemiology. Expert Opin Ther Targets 13(2): 163-173.
6. Zoccali C, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, et al. (2002) Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. J Am Soc Nephrol 13(1): 134-141.
7. Shen YY, Charlesworth JA, Kelly JJ, Loi KW, Peake PW (2007) Up-regulation of adiponectin, its isoforms and receptors in end-stage kidney disease. Nephrol Dial Transplant 22(1): 171-178.
Adipocytokines leptin and adiponectin, and measures of malnutrition-inflammation in chronic renal failure: is there a relationship? J Ren Nutr 18(4): 332-337.

22. Yamamoto K, Kiyohara T, Murayama Y, Kihara S, Okamoto Y, et al. (2005) Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn’s disease. Gut 54(6): 789-796.

23. Foley RN, Parfrey PS, Sarnak MJ (1998) Epidemiology of cardiovascular disease in chronic renal disease. J Am Soc Nephrol 9(12 Suppl): S16-S23.

24. Foley RN, Murray AM, Li S, Herzog CA, McBean AM, et al. (2005) Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. J Am Soc Nephrol 16(2): 419-495.

25. Iwashima Y, Katsuura T, Ishikawa K, Ouchi N, Ohishi M, et al. (2004) Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 43(6): 1318-1323.

26. Iwashima Y, Horio T, Kumada M, Suzuki Y, Kihara S, et al. (2006) Adiponectin and renal function, and implication as a risk of cardiovascular disease. Am J Cardiol 98(12): 1603-1608.

27. Schiepers G, Westerling F, Brandenburg V, Ketteler M (2007) Inhibitors of calcification in blood and urine. Semin Dial 20(2): 113-121.

28. Tsao TS, Murray HE, Hug C, Lee DH, Lodish HF (2002) Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). J Biol Chem 277(33): 29359-29362.

29. Kobayashi Y, Udagawa N, Takahashi N (2009) Action of RANKL and OPG for osteoclastogenesis. Crit Rev Eukaryot Gene Expr 19(1): 61-72.

30. Cannape-Yared MH, Fares E, Semaan M, Khalife S, Jambart S (2006) Circulating osteoprotegerin is correlated with lipid profile, insulin sensitivity, and adiponectin and sex steroids in an ageing male population. Clin Endocrinol (Oxf) 64(6): 652-658.

31. Pritzker LB, Scatena M, Giachelli CM (2004) The role of osteoprotegerin and tumor necrosis factor-related apoptosis-inducing ligand in human microvascular endothelial cell survival. Mol Biol Cell 15(6): 2834-2841.

32. Malvankar UM, Scatena M, Suchland KL, Yun TJ, Clark EA, et al. (2000) Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells. J Biol Chem 275(28): 20959-20962.

33. Petras D, Fortunato A, Soffiati G, Brendolan A, Bonello M, et al. (2005) Sequential convective therapies (SCT): a prospective study on survival factor for endothelial cells. J Endocrinol Investig 28(5): 482-488.

34. Eloit S, Schneditz D, Cornells T, Van Biesen W, Gloor G, et al. (2016) Protein-Bound Uremic Toxin Profiling as a Tool to Optimize Hemodialysis. PloS One 11(1): e0147159.

35. Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT (2006) Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes 55(1): 249-259.