Inflammatory cytokines and fibrotic factors in diabetic nephropathy

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Declaration

Research grants, Consultancy and Advisory boards

• AstraZeneca
• Baxter Biosciences
• Boehringer Ingelheim
• GSK
• MedImmune
• Novartis
• Rigel Pharmaceuticals
Outline

Diabetic nephropathy:
- The clinical challenge
- Inflammatory cytokines: MCP-1
- Fibrotic factors: TGF-β1, CTGF, CCL18
- Biomarker studies
- Clinical trial
- Different clinical phenotypes
Diabetic Microvascular Complications

• retinopathy, nephropathy and neuropathy
• strong clinical association
• debilitating
Progression of diabetic kidney disease

- **Hypertension**
- **Glucose ↑↑**
- **Inflammation**
- **Scarred kidney**
- **Kidney failure**

**Background**
Diabetic nephropathy
the clinical challenge
25-50% of patients on dialysis & transplantation

Renal failure

Proteinuria

Microalbuminuria

“normal”
progression of diabetic nephropathy

• large number of diabetic patients still have \textit{progressive} renal disease,

• even with optimal medical treatment
Inflammation in diabetic nephropathy

- **Traditional theory**
  - deposition of extracellular matrix resulting in fibrosis

- **Unexpected finding**
  - increased in number of macrophages
    - Experimental models
    - Renal biopsies of patients
Inflammation in diabetic nephropathy

Robert I. Menzies, Frederick W. Tam, Robert J. Unwin, Matthew A. Bailey
Recruitment of inflammatory cells to the kidney

- Rolling
- Activation
- Adhesion
  - integrins
- Transmigration

selectins
chemokines e.g. MCP-1
cell adhesion molecules

endothelium
lumen of blood vessels
kidney tissue
chemokines
## Classification of chemokines

<table>
<thead>
<tr>
<th>Subfamilies</th>
<th>Responding cells</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXC (ELR)</td>
<td>Neutrophils</td>
<td>IL-8</td>
</tr>
<tr>
<td>CXC (-ELR)</td>
<td>Mϕ</td>
<td>IP-10</td>
</tr>
<tr>
<td>CC</td>
<td>Mϕ</td>
<td>MCP-1</td>
</tr>
<tr>
<td>C</td>
<td>T cells</td>
<td>lymphotactin</td>
</tr>
<tr>
<td>CX3C</td>
<td>Mϕ</td>
<td>fractalkine</td>
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</table>
Monocyte chemoattractant protein-1 (MCP-1) also known as CCL2

- a chemoattractant to macrophage & T cells
- activate monocyte/macrophages
  - production of IL-1
  - superoxide production
- increase in urine of patients with diabetic nephropathy (Saitoh A et al Nephron 1998)
- secreted by glucose stimulated mesangial cells (Ihm CG et al Nephron 1998)
- renal biopsies from patients (Wada T et al Kidney Int 2000)
Production of MCP-1 by human mesangial cells

Glucose & Mechanism stretch

Effects of advanced glycation endproduct (AGE) on MCP-1 expression in mesangial cells

Yamagishi, S.-i. et al. J. Biol. Chem. 2002;277:20309-20315
MCP-1

Clinical studies
Increased urinary MCP-1

- Combined Type 1 & 2
  - Increased in micro and macroalbuminuric patients
  - Correlated with serum glycated albumin
- Type 2 diabetic
  - 3 fold increase in microalbuminuric patients
  - 5 fold increase in macroalbuminuric patients
Hypothesis

• MCP-1 is important in progression of diabetic nephropathy

Aim

• Assess urinary levels of MCP-1 at different stages of diabetic nephropathy
• Correlate urinary MCP-1 with subsequent changes in albuminuria and renal function

Tam, Riser et al Cytokine 2009
Increased urinary MCP-1 in diabetic nephropathy

- 3 fold $\uparrow$ in diabetic nephropathy (p<0.01)
- 4 fold $\uparrow$ in diabetic retinopathy (p<0.0005)

Tam, Riser, Frankel et al *Cytokine* 2009
Urinary MCP-1 is prognostic of fall in GFR

R = 0.61  **p < 0.0001

Urinary MCP-1
prognostic of ↓ renal function (eGFR, estimated glomerular filtration rate) over 6 years follow up

Tam, Riser, Frankel et al  *Cytokine* 2009
Urinary MCP-1 a more useful prognostic marker than proteinuria in patients with established diabetic nephropathy

Tam, Riser, Frankel et al  *Cytokine* 2009
Validation studies

• Study of 56 patients over 2.5 years (Brazil):

• 83 patients with diabetic nephropathy (Canada):
  Measured 7 urinary biomarkers
  Urinary MCP-1 and TGF-β as independent predictor of fall in kidney function over 2.1 years.

• Study of 380 patients from a USA clinical trial.
Selection of cases and controls from overall cohort.
Validation studies: urinary MCP-1 is a prognostic biomarker of decline of kidney function in diabetic kidney disease

- Action to Control Cardiovascular Risk in Diabetes (ACCORD) Trial
- Type 2 diabetes
- Matching baseline key clinical features (include age, sex, race, eGFR, baseline urine albumin/creatinine ratio
- 190 patients with $\geq 40\%$ sustained eGFR decline over 5 years follow-up vs 190 patients with $\leq 10\%$ decline
- Multiplex platform of biomarkers (MCP-1, IL-18, KIM-1, YKL-40) in baseline and 24 months
- Only Urinary MCP-1/creatinine ratio is higher in the patients in patients with sustained decline of kidney functions

Inhibition of MCP-1 reduced experimental diabetic nephropathy (DN)

• MCP-1 antagonist ↓ progression of murine DN. Kanamori H et al BCCR 360 (2007) 772-7
Clinical trial of inhibitor of MCP-1 receptor (CCR2)

- Patients with type 2 diabetes and albuminuria
- Randomised double-blind placebo-controlled clinical trial
- 78 European renal centres, including UK
- Key inclusion criteria:
  - Type 2 diabetes
  - 18-75 years old
  - Proteinuria (first morning void urine albumin/creatinine ratio 100-3000 mg/g)
  - eGFR ≥ 25 ml/min/1.73 m²
  - ACE inhibitor or ARB for at least 8 weeks
- Treatment for 52 weeks
- Placebo
- CCX140-B (CCR2 inhibitor) 5 mg once daily
- CCX140-B 10 mg once daily

De Zeeuw et al Lancet Diabetes Endocrinol 2015; 3:687
Clinical trial of CCR2 inhibitor

Treatment with 5 mg/day of CCX140-B resulted in 18% reduction in albuminuria (v 2% in placebo group v 11% in 10 mg/day group)

De Zeeuw et al Lancet Diabetes Endocrinol 2015; 3:687
Interaction between MCP-1 and its receptors

- Blocking CCR2 is not the same as inhibiting MCP-1

(Tam & Ong, 2019, Nephrol Dial Transplant)
Summary: MCP-1

- Strong clinical association between diabetic retinopathy and nephropathy
- Unmet medical need despite standard of care with glycaemic control, treatment of hypertension and use of ACEI/ARB
- Urinary MCP-1 is prognostic biomarker of progression of diabetic nephropathy
- MCP-1 and its receptors are novel therapeutic targets:
  – receptor CCR2 (phase 2 clinical trial)
Inflammation and fibrosis in diabetic nephropathy

Angiotensin II → MCP-1 → inflammation → Renal failure

Glucose → MCP-1 → inflammation

Hypertension → MCP-1 → inflammation

TGF-β → CTGF → Extracellular matrix → scarring
Fibrotic factors

• Transforming growth factor (TGF)β1
• Connective tissue growth factor (CTGF)
  – also know as CCN2
• CCL18

CCN=CYR61/CTGF/NOV
TGF-β

• Increased expression in renal biopsy of patients
• Increased in experimental model
• Induce by
  – high glucose
  – AGE
  – Angiotensin II
  – mechanical stretch
• Hypertrophy
• Matrix deposition
• Stimulate production of CTGF
Effect on cholecalciferol treatment on diabetic nephropathy

Kim MJ et al. Kidney Int 2011;80:851-60
Reduction of albuminuria and TGF-β1 - following replacement of vitamin D

Kim MJ et al. Kidney Int 2011;80:851-60
Connective tissue growth factor (CTGF)

- Also known as CCN2
- Mediates some of the effects of TGF-β in deposition of extracellular matrix (Wahab & Mason Cur Opinion Nephrol Hypertension 2004)
- Produced by mesangial and tubular epithelial cells (Wahab NA et al Biochem J 2001)
- Increased expression in all stages of diabetic nephropathy in patients (Wahab NA et al Biochem J 2001)
- Increased amounts in urine of patients with diabetic nephropathy (Riser BL et al Kidney Int 2003)
Expression of glomerular CTGF during the development of DN

Wahab & Mason
Detection of urinary CTGF (CCN2)

• CCN2 measured by ELISA
  – Capture Ab: affinity purified goat anti-CTGF (Santa Cruz)
  – Detection Ab: polyclonal rabbit Ab agonist 20 kDa C-terminal fragment
  – Detect whole molecule and also the C-terminal fragments
Increased urinary CTGF (CCN2) in diabetic nephropathy

Diabetic patients

 severity of albuminuria

Tam, Riser et al Cytokine 2009
CTGF-Key points

CTGF/CCN2
• Mediate downstream effect of TGF-β1
• Knockdown of CTGF reduced the severity of experimental DN

Urinary CTGF
• is an early marker of diabetic nephropathy.
• elevated in patient with micro- and macroalbuminuria
• Prognostic of rise in microalbuminuria over 1 year
CCL18
CCL18/PARC/MIP-4

- Pulmonary and activation regulated chemokine (PARC) is produced by monocytes/macrophages and dendritic cells
- Chemotactic for both naive and activated T-cells, but not granulocytes or monocytes
- Stimulate collagen production by
  - a TGF-β independent pathway
  - recruitment of pro-fibrotic lymphocytes
- In patients receiving long term peritoneal dialysis, increased dialysate CCL18, correlate with amount of glucose in dialysate

2. Luzina et al: 2006 J Cell Physiol 206, 221-228
4. Ahmad et al (unpublished results)
Prospective observational study

- Diabetic patients (n=101, including 10 with type 1, 91 with type 2 diabetes)
Increased urinary CCL18 in overt diabetic nephropathy

Qureshi et al 2007 J Am Soc Nephrol 18;325A
Synergistic Effect of CCL18 and high glucose concentration on extracellular matrix production

Human tubuloepithelial cell line

N: normal glucose concentration
A: glycated albumin
M: mannitol (osmolarity control)
H: high concentration of glucose

Montero RM et al
BMC Nephrology 2016; 17:139
Application to diabetic nephropathy

- Glucose $\uparrow \uparrow$
- Advanced glycation end products
- Hypertension
- Angiotensin II

- MCP-1
- Other cytokines

- Inflammation
- Renal failure
- Scarring

- TGF-\(\beta\)
- CTGF

- Extracellular matrix

- CCL18
Defining phenotypes of diabetic nephropathy
Principal component analysis of 30 clinical features of diabetes/CKD patients

Montero et al
Scientific Reports
Jan 2018

New clinical approach

Ongoing longitudinal follow-up (>12 years) to study the prognostic values of clinical phenotypes & cytokines
Conclusion

• Metabolic problems (diabetes and obesity) are a major cause of chronic kidney disease and kidney failure
• Increased inflammatory cytokines (MCP-1) and fibrotic cytokines/factors (TGFβ, CTGF, CCL18)
• Non-invasive biomarkers
• Therapeutic targets
Acknowledgement

- Andrew Frankel
- Ashfaq Qureshi
- Athula Herath
- Ehsanollah Esfandiari
- Gurjeet Bhangal
- Hsiu Lye Yap
- Marco Bueter
- Min Jeong Kim
- Nadia Wahab
- Rosa Montero
- Sukhpreet Singh Dubb
- Prof Anne Dornhorst
- Prof Carel le Roux
- Prof Charles Pusey
- Prof Karim Meeran
- Prof Roger Mason

- Ken and Mary Minton Chair of Renal Medicine
- Diamond Fund
- Imperial College Healthcare NHS Trust Charity Fund
- National Institute for Health Research (NIHR) Biomedical Research Centre
- Kidney Research UK
- Medical Research Council

- Chicago Medical School
  - Prof. Bruce Riser