Uncomplicating diabetes: Interactions between metabolic and haemodynamic signalling pathways in the pathogenesis of diabetic complications
Acknowledgment

The content of this slide deck summarizes the key points presented during the EASD Claude Bernard Prize Lecture by Professor Mark E. Cooper at the 52nd Annual Meeting of the European Association for the Study of Diabetes, September 12–16 2016, Munich, Germany
Contents

• Factors involved in diabetic neuropathy
• ACE inhibition
• Impact of AGE modification
• Role of ROS in neuropathy
• CDA₁ and fibrogenesis
• The legacy effect
• Epigenetic reprogramming
• Summary

ACE: angiotensin converting enzyme
AGE: advanced glycation end-products
ROS: reactive oxygen species; Ang II: angiotensin II
CDA₁: CDA1: cell division autoantigen 1
A physician’s subject of study is the patient, but if **clinical observation** teaches us to know the form and course of diseases it **cannot** suffice to make us understand their nature

Claude Bernard

*An Introduction to the Study of Experimental Medicine* (1965)
Pathogenesis, prevention and treatment of diabetic nephropathy

Metabolic
- AGEs
- Cytokines, TGFβ, PKC, VEGF, etc.

Haemodynamic
- ROS
- Ang II, ET₁

↑Matrix
↑Vascular permeability dysfunction

Pathogenic mediators
Signalling pathways

Post-translational modifications

TGFβ: transforming growth factor β
VEGF: vascular endothelial growth factor

Pathogenesis, prevention and treatment of diabetic nephropathy

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Treatment with an ACE inhibitor can induce remission of proteinuria

- Forty-two patients were randomised to receive perindopril, slow-release nifedipine or placebo
- Doses in the first three months were titrated to decrease diastolic BP by ≥5 mmHg and patients were followed up for a minimum of 24 months
- In long-term therapy, perindopril was more effective than nifedipine or placebo in delaying the progression of diabetic neuropathy (p<0.03)\(^1\)

Additionally, in an ApoE KO mouse model, ACE inhibition inhibited diabetes-accelerated atherosclerosis\(^2\)

Pathogenesis, prevention and treatment of diabetic nephropathy

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The impact of AGE modifications

An excess of dicarbonyls, an intermediate reactive species in the production of AGEs, has been shown to mimic the complications of diabetes

Modifications caused by AGE compounds can cause the following pathological complications in proteins:

- Alter surface charge
- Structure/conformation
- Function/affinity
- Impaired turnover
- Modify interactions
- Trigger signalling
- Generate ROS

Lysine-AGE
Arginine-AGE
Histidine-AGE
Cysteine-AGE
Blocking both AGEs and RAAS produced an additive benefit on albuminuria

**Methods**

- Streptozocin-induced diabetic spontaneously hypertensive rats were randomised to receive no treatment, perindopril, aminoguadine or a combination of these agents
- The impact on AER was measured over 32 weeks

**Results**

- No treatment resulted in increased AER
- Both perindopril and aminoguadine significantly reduce AER versus no treatment (p<0.01)
- Combination treatment reduced AER to a significantly greater degree than either monotherapy (p<0.01)

**Conclusion**

- Superior renoprotection was achieved by blocking both the AGE and RAAs pathways compared with each pathway alone
- This could be a useful therapeutic strategy for preventing or reducing progressive diabetes-related renal injury


RAAS: renin-angiotensin-aldosterone system

AER: albumin excretion rate
Ligand-dependent activation of RAGE in diabetes can result in complications

- In ApoE KO mice, deletion of RAGE has been found to prevent the development of diabetes-associated atherosclerosis

**Non-AGE ligands**: S₁₀₀, HMGB₁, AβP

**AGE-ligands**: Lysine-CEL, Arginine-MGH₁, Histidine-AGE, Cysteine-AGE

**Complications**:
- ROS↑
- NFKB↑
- PKC↑
- RAAS↑
- TGFβ↑
- Glyoxalase↓

HMBG1: High mobility group box 1 protein; AβP: Amyloid beta protein; CEL: N-epsilon-(Carboxyethyl) MGH1: methylglyoxal-derived hydroimidazolone N5-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine

RAGE: receptor for advanced glycation end-products; NFKB: nuclear factor KB

Pathogenesis, prevention and treatment of diabetic nephropathy

**Metabolic**
- AGEs

**Haemodynamic**
- ROS
- Ang II

**Pathogenic mediators**
- Cytokines, TGFβ, PKC, VEGF, etc.

**Signalling pathways**
- ↑Matrix
- ↑Vascular permeability dysfunction

Potential sources of superoxide generation in diabetes

- Xanthine oxidase
- NAD(P)H oxidases (Nox)
- Uncoupled NOS
- Lipoxygenase
- Cyclo-oxygenase
- ER stress
- AGE/RAGE

NAD(P)H: nicotinamide adenine dinucleotide phosphate
Nox: NAD(P)H oxidase; NOS: nitrous oxide
ER: endoplasmic reticulum

Nox activity and expression is increased by diabetes in vulnerable tissues

\[ \text{DIABETES} \]

- ↑Ang II
- ↑PKC
- ↑RAGE/TLR
- ↑Growth factors (e.g. PDGF)
- ↑Cytokines (e.g. TNFα)
- ↑Stress/strain
- ↑Lipids (e.g. LPA, oxLDL)
- ΔREDOX state

\[ \text{Superoxide} \rightarrow H_2O_2 \quad OH \quad ONOO^- \quad HOCI \quad \text{ROS} \]

DUOX: dual oxidase 1

TLR: toll-like receptor; PDGF: platelet-derived growth factor
TNFα: tumour necrosis factor α; LPA: lipoprotein A
oxLDL: oxidised low-density lipoprotein

Optimal inhibition of Nox with GKT137831

- A mouse model of diabetic neuropathy, was used to determine the impact of Nox deletions on renal function\(^1\)
- Nox4 was found to be the main source of renal ROS and deletion of this gene conferred a renoprotective effect\(^1\)
- Administration of the Nox1/4 inhibitor GKT137831 in mice without the Nox deletion replicated these renoprotective effects\(^1\)

### Renal function\(^1\)
- Deletion of Nox4 reduced the levels of albuminuria in the mouse model
- Similarly, treatment with GKT137831 protected against the development of albuminuria in the control animals

### Renal injury and ROS\(^1\)
- Levels of the oxidative stress marker nitrotyrosine were increased in the diabetic mice
- However, Nox4 deletion or treatment with GKT137831 reduced nitrotyrosine accumulation in the glomeruli

- A randomised, controlled trial of GKT137831 in patients with T1DM and macro/microalbuminuria is planned, but the design of the study is yet to be determined\(^2\)

Pathogenesis, prevention and treatment of diabetic nephropathy

CDA$_1$ and fibrogenesis

- CDA$_1$ enhances TGFβ signalling and renal expression of CDA$_1$ is elevated in mouse models of diabetes
- Deletion of CDA$_1$ and CDA$_1$BP attenuated glomerular and tubulointerstitial injury compared with wild-type mice

Inhibition of the interaction between CDA$_1$ and CDA$_1$BP

- The putative CDA$_1$ inhibitor CHA-061 is being investigated as a possible treatment aimed at reducing the damage caused by diabetic neuropathy.

The legacy effect

Metabolic

Haemodynamic

AGEs

ROS

Ang II

Post-translational modifications

Direct signalling

Compositional change
(hypertrophy, scarring, drop-out, vaso-regression, etc.)

Epigenetic reprogramming

Long-term legacy
Impact of the legacy effect

The STENO-2 trial aimed to study the potential long-term impact of 7.8 years of intensified, multifactorial intervention vs. conventional treatment in patients with T2DM (N=160)

A significantly greater number of patients who received standard therapy died during follow-up compared with intensive therapy (HR 0.55 [95% CI: 0.36, 0.83], p=0.005)

At 21.2 year follow-up patients in the intensive-therapy group survived for a median of 7.9 years long than standard therapy

The legacy effect

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Ang II
Epigenetic reprogramming

“The structural adaptation of chromosomal regions so as to store regions so as to store, retain, and recall past experiences in a way to shape present and future behaviour.”

This includes:

• DNA methylation
• Histone post-translational
• RNA-based mechanisms

Bird A. Nature 2007;447:396–8
Genetic transcription is partly regulated by chemical modification, such as methylation, of the unstructured ends of histone proteins.

Transient hyperglycaemia can also result in epigenetic changes that may result in diabetic complications.
Transient hyperglycaemia and epigenetic changes

**Transient hypoglycaemia model:** Primary bovine or human aortic endothelial cells were incubated in high glucose and then returned to media with a normal glucose concentration.

- Transient hyperglycaemia resulted in the persistent methylation of the histone H3K4.
- This led to the upregulation of the NFKB p65, which has a prominent role in inflammatory pathways.

- These epigenetic changes were also confirmed in a non-diabetic mouse model.
- Mice exposed to hyperglycaemia for 6h via pancreatic insulin clamp demonstrated increased H3K4 methylation levels and NFKB p65 expression.

Actions and consequences

• Efforts today can have result in long-term improvements
• Conversely, mistakes can have ramifications for years to come
• Hyperglycaemic excursions have a pathogenic role:
  – Variations in hyperglycaemia
  – Post-prandial hyperglycaemia
  – Complications can occur without HbA1c
  – Vascular complications may also be present in pre-diabetes
Abbreviations

- AβP: Amyloid beta protein
- ACE: angiotensin converting enzyme
- AER: albumin excretion rate
- AGE: advanced glycation end-products
- ApoE: Apolipoprotein E
- Ang II: angiotensin II
- C: control
- CEL: N-epsilon-(Carboxyethyl)
- CDA1: cell division autoantigen 1
- CDA1BP: cell division autoantigen 1 binding protein
- D: diabetes
- DUOX: dual oxidase 1
- ER: endoplasmic reticulum
- ET1: endothelin 1
- GKT: GKT137831
- GTP: guanosine triphosphate
- HG: high glucose
- HMBG1: High mobility group box 1 protein
- KO: knockout
- LG: low glucose
- LPA: lipoprotein A
- MGH1: methylglyoxal-derived hydroimidazolone Nδ-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine
- NAD(P)H: nicotinamide adenine dinucleotide phosphate
- NFκB: nuclear factor KB
- NOS: nitrous oxide
- Nox: NAD(P)H oxidase
- oLDL: oxidised low-density lipoprotein
- PDGF: platelet-derived growth factor
- PKC: protein kinase
- pSMAD: Mothers against decapentaplegic homolog protein
- RAAS: renin-angiotensin-aldosterone system
- RAC: Ras-related C3 botulinum toxin substrate
- RAGE: receptor for advanced glycation end-products
- ROS: reactive oxygen species
- SHR: spontaneously hypertensive rat
- T1DM: type 1 diabetes mellitus
- TGFβ: transforming growth factor β
- TGFβR: TGFβ receptor
- TLR: toll-like receptor
- TNFα: tumour necrosis factor α
- VEGF: vascular endothelial growth factor