GLP-1 analogues and diabetic microangiopathy

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_Vall d’Hebron Research Institut. Barcelona._
Diabetic complications represent a significant economic burden in all western health systems.

- A 2- to 4-fold increase in cardio-vascular mortality
- The leading cause of new cases of end stage renal disease
- The leading cause of new cases of blindness in working-aged adults
- The leading cause of nontraumatic lower extremity amputations
DR IS AN INDEPENDENT RISK FACTOR FOR CVD

Coronary artery disease
(Cheung et al. Diabetes Care 2007)

Heart Failure
(Cheung et al. J Am Coll Cardiol 2008)

Stroke
(Cheung et al. Stroke 2007)

Cardiovascular mortality
(Juutilainen et al. Diabetes Care 2007)
Clandestine ischemia is the presence of myocardial perfusion defects in the absence of both angina and ST-segment depression during the exercise test.

Prevalence: 10-fold higher (24.4 vs. 2.4%; p<0.01) in T2DM than in nondiabetic controls matched by age and gender.

The presence of defects in myocardial perfusion was independently associated with the presence of diabetic retinopathy and male gender.

Odds ratio 11.7
[CI95%: 3.7-37]

DR

Positive SPECT

Cardiovasc Diabetol 2011; 21(1):9
Overview of diabetic treatment based on GLP-1 analogues and its potential benefit effects in cardiovascular disease.

GLP-1R agonists effects in diabetic microangiopathy:
- Cardiac microvascular injury
- Diabetic nephropathy
- Diabetic neuropathy
- Diabetic retinopathy
GLP-1 AND DIABETIC MICROANGIOPATHY

-Overview of diabetic treatment based on GLP-1 analogues and its potential benefit effects in cardiovascular disease.

-GLP-1R agonists effects in diabetic microangiopathy:
  - Cardiac microvascular injury
  - Diabetic nephropathy
  - Diabetic neuropathy
  - Diabetic retinopathy
GLP-1 has multiple direct effects on human physiology

- **Insulin-secretion (glucose-dependent)**
- **Beta-cell mass**
- **Glucagon secretion (glucose-dependent)**
- **Hepatic glucose output**
- **Pancreas**
- **GI tract**
- **SNC**
- **Stomach**
- **Hearth and arteries**

- **Satiety**
- **Neuroprotection**
- **Gastric emptying**
- **Cardiovascular protection**
**Exendin-4 has an anti-hypertensive effect in salt-sensitive mice model**

In db/db mice, the urinary sodium excretion was delayed and BP was elevated in response to a high-salt load. These effects were attenuated by exendin-4.

Exendin-4 inhibits angiotensin II induced increase of systolic blood pressure.
GLP-1 decrease cardiac myocyte apoptosis and increase glucose uptake
GLP-1 AND DIABETIC MICROANGIOPATHY

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  - Diabetic nephropathy
  - Diabetic neuropathy
  - Diabetic retinopathy
GLP-1R agonist protects against cardiac microvascular injury in diabetes

STZ-induced diabetic rats

Exenatide treatment maintained cardiac microvascular integrity

Control  DM + vehicle  DM + exenatide

Scanning electron microscopy

Exenatide treatment protected cardiac microvascular barrier function

Control  DM + vehicle  DM + exenatide

Lanthanum nitrate

Both effects were more pronounced with exenatide than when using insulin

Wang et al. Diabetes 2013
GLP-1 attenuated high-glucose–induced oxidative stress in cardiac microvascular endothelial cells

Dihydroethidine staining to detect superoxide

GLP-1 decreased high-glucose–induced apoptosis in cardiac microvascular endothelial cells

Downstream inhibition of Rho through cAMP/PKA mediated pathway.

Wang et al. Diabetes 2013
Exenatide treatment improves cardiac glucose uptake in diabetes

18F-FDG

Wang et al. Diabetes 2013
GLP-1 AND DIABETIC MICROANGIOPATHY

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- Cardiac microvascular injury
- Diabetic nephropathy
- Diabetic neuropathy
- Diabetic retinopathy
Exendin-4 treatment increases GLP-1R expression and ameliorates diabetic nephropathy
Beneficial effects of exendin-4 treatment in diabetic nephropathy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$db$ Control</th>
<th>$db+0.5$ Exendin-4</th>
<th>$db+1.0$ Exendin-4</th>
<th>$dm$ Control</th>
<th>$dm+1.0$ Exendin-4</th>
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<td>Kidney/body weight (%)</td>
<td>0.62 ± 0.19</td>
<td>0.54 ± 0.15</td>
<td>0.64 ± 0.19</td>
<td>0.69 ± 0.14</td>
<td>0.64 ± 0.06</td>
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<td>BUN (mg/dl)</td>
<td>24.8 ± 6.8</td>
<td>26.0 ± 3.2</td>
<td>24.0 ± 2.6</td>
<td>19.1 ± 3.9</td>
<td>21.4 ± 2.3</td>
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<td>Creatinine (mg/dl)</td>
<td>0.08 ± 0.03</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
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<td>Urine volume (ml)</td>
<td>22.7 ± 6.0</td>
<td>24.3 ± 9.0</td>
<td>13.7 ± 4.3</td>
<td>1.8 ± 1.4</td>
<td>0.7 ± 0.7</td>
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<td>Ccr (ml/min)</td>
<td>1.14 ± 0.32</td>
<td>0.69 ± 0.37</td>
<td>0.52 ± 0.23</td>
<td>0.33 ± 0.12</td>
<td>0.29 ± 0.28</td>
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<td>24-h urinary albumin (μg)</td>
<td>428 ± 191</td>
<td>306 ± 189</td>
<td>121 ± 70</td>
<td>21 ± 19</td>
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$^a$BUN, blood urea nitrogen; Ccr, creatinine clearance.

$^b$P < 0.01, $^c$P < 0.001 compared to $db/db$ controls.
Exenatide ameliorates renal injury without lowering blood glucose levels

STZ-induced diabetic rats

Exenatide ameliorates renal injury through direct effect on the GLP-1R in the kidney. This effect is mainly accounted for its anti-inflammatory action.
Liraglutide protects against oxidative stress and albuminuria in STZ-induced diabetic rats

These effects are via protein kinase A-mediated inhibition of renal NAD(P)H oxidases

Hendarto et al. Metabolism 2012
Effect of liraglutide in the synthesis of matrix proteins in the kidney

Hendarto et al. Metabolism 2012
GLP-1 AND DIABETIC MICROANGIOPATHY

Overview of diabetic treatment based on GLP-1 (incretin mimetics) and its potential benefit effects in cardiovascular disease.

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- Cardiac microvascular injury
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- **Diabetic neuropathy**
- Diabetic retinopathy
Beneficial effects of exendin-4 on experimental polyneuropathy

STZ–induced diabetic mice

Exendin-4 administration

- Improved the reduced sensory perception and delayed NCV in diabetic mice
- Ameilorates the loss of nerve fibers in the epidermis of diabetic mice
GLP-1 AND DIABETIC MICROANGIOPATHY

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GLP-1R agonist effects in diabetic microangiopathy:
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- Diabetic nephropathy
- Diabetic neuropathy
- Diabetic retinopathy
Up to 30% of diabetic patients present some degree of DR
10% with advanced disease (sight-threatening DR)
Diabetic complications: neuropathy, blindness, and renal failure

**No DR**

**NPDR**
- VEGF
- Proinflammatory Cytokines

**PDR**
- Capillary Occlusion
- Hypoxia
- DME
- BRB breakdown
- Neovascularization

**Diabetic pathways**
- Polyl pathway
  - NADPH → NADP^+ → Sorbitol → Fructose
- Hexosamine pathway
  - GFAT → Glucosamine-6-P → UDP-GlcNAc
  - Glu → Gln
- Protein kinase C pathway
  - NADH → NAD^+
  - DHAP → ω-Glycerol-P → DAG → PKC
- AGE pathway
  - Methylglyoxal → TAGEs

**BRB breakdown**

**VEGF**
TREATMENT OF DIABETIC RETINOPATHY

Tight control of glycemia and blood pressure

No DR

NPDR

DME

PDR

- Laser photocoagulation
- Intravitreal injections of corticosteroids or anti-VEGF
- Vitreo-retinal surgery
Retinal neurodegeneration

Antedates and participates in the microcirculatory abnormalities

Polyol pathway
NADPH → NADP⁺ → ↑Sorbitol → NAD⁺ → NADH → ↑Fructose

Hexosamine pathway
GFAT → ↑Glucosamine-6-P → ↑UDP-GlcNAC

Protein kinase C pathway
NAD⁺ → ↑GAPDH → ↑O₂⁻ → ↑DHAP → ↑α-Glycerol-P → ↑DAG → ↑PKC

AGE pathway
↑Methylglyoxal → TAGEs

Capillary Occlusion

Hypoxia

↑VEGF
↓PEDF

neovascularization

Diabetic complications: for example, neuropathy, blindness, and renal failure

No DR

NPDR

DME

PDR

Capillary Occlusion

Hypoxia

↑VEGF
↓PEDF
Neurodegeneration as an early event in DR


Neurodegeneration as an early event in DR


Neurodegeneration as an early event in DR


Carrasco E, Hernández C, de Torres I, Farrés J, Simó R. Lower costistatin expression is an early event in the human diabetic retina and is associated with apoptosis and glial activation. Mol Vis 2008;14:1496-502


Neurodegeneration in retinas from diabetic donors

Reactive gliosis

Absence of microvascular abnormalities

Apoptosis

*Diabetes Care* 2007; *Diabetes Care* 2008
*Mol Vis* 2008; *Diabetologia* 2009

Neurodegeneration is an early event in the pathogenesis of DR
mfERG abnormalities predict which retinal locations will develop microangiopathy in the near future

Han et al. IOVS 2004
Bearse et al. Prog Retin Eye Res 2006
Ng et al. IOVS 2008
Harrison et al. IOVS 2011
Mechanisms linking neurodegeneration and microangiopathy

- Polyol pathway
- Hexosamine pathway
- DAG-PKC
- Oxidative stress
- Proinflammatory cytokines
- AGE upregulation
- RAS activation

Early microvascular impairment

Neurovascular unit impairment

Neurodegeneration

↑ Glutamate/NMDA receptors (excitotoxicity)

Loss of neuroprotective Factors

↑ Epo
↑ VEGF

BRB breakdown
Vasoregression
Impaired hemodynamic response

Neuron death
Glial dysfunction

Low number and dysfunctional EPCs

SST, CST, PEDF, IRBP
NTs (↑ ProNGF/NGF)

Adapted from Simó R et al. Trends Endocrinol Metab 2014
Neurodegeneration as an early event in DR

International competitive projects

EFSD European Research Programme in Micro and Macrovascular Complications of Diabetes (2011)
“Neurodegeneration as an early event in the pathogenesis of DR. Study of involved mechanisms and new therapeutic strategies”
100,000 €

Sequential characterization of neurodegeneration in db/db mice

EFSD Mental Health and Diabetes Programme (2013)
“Retinal neurodegeneration in type 2 diabetes as biomarker for Alzheimer’s disease”. The eye as a window of brain disease.
250,000 €

EURODIAL
European consortium for the study of Diabetes as an accelerator of Alzheimer’s disease (Horizon 2020)

7th FP-HEALTH-2011
Investigator-driven Clinical Trials to Reduce Diabetes complications
6M€

Neurodegeneration as an early event in the pathogenesis of DR. A multicentric, prospective, phase II-III, open randomized controlled trial to assess the efficacy of neuroprotective drugs administered topically to prevent or arrest DR

Consortium: EUROCONDOR
Characteristic features of neurodegeneration in db/db mouse model

Reactive gliosis (GFAP) (8 w) Apoptosis (TUNEL)

This structural changes are associated with typical ERG abnormalities

Diabetes-induced neurodegeneration is associated with an accumulation of glutamate due to a reduction of GLAST and glutamine synthetase.

GLAST: key molecule for the clearance of glutamate from extracellular space, thus reducing the excitotoxicity.
Neurodegeneration as an early event in DR

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**Consortium: EUROCONDOR**
EUROCONDOR
European Consortium for the Early Treatment of Diabetic Retinopathy

Ophthalmologists

José Cunha-Vaz (Portugal)
Jacob Grauslund (Denmark)
Pascale Massin (France)
Francesco Bandello (Italy)
Edoardo Midena (Italy)
Gabriele Lang (Germany)
Simon Harding (UK)
Peter Scanlon (UK)
Jonathan Gibson (UK)
Catherine Egan (UK)
José García-Arumí (Spain)

Diabetologists/Basic Researchers

Deborah Burks (Spain)
Angela M. Valverde (Spain)
Massimo Porta (Italy)
Rafael Simó (Spain) [Coordinator]

http://eurocondor.eu
## Visits summary

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**EUROCONDOR CLINICAL TRIAL**

End of the recruitment period: 31th October 2013
First results (year follow-up): November 2014
Final report: January 2016
European Consortium for the Early Treatment of Diabetic Retinopathy

Visit our website: http://eurocondor.eu
GLP-1 has multiple direct effects on human physiology

- Insulin-secretion (glucose-dependent)
- Beta-cell mass
- Glucagon secretion (glucose-dependent)
- Hepatic glucose output

Pancreas

- Satiety
- Neuroprotection

SNC

Liver

Stomach

Gastric emptying

GI tract

Hearth and arteries

Cardiovascular protection

* There are several ongoing clinical trials using GLP-1R agonists for preventing AD
NEW THERAPEUTIC APPROACH OF DR

GLP-1 agonists as neuroprotective agents in DR

Tight control of glycemia and blood pressure

No DR

NPDR

Neurodegeneration as a new pathogenic factor

DME-CSDME

PDR

- Laser photocoagulation
- Intravitreal injections of corticosteroids or anti-VEGF
- Vitreo-retinal surgery
GLP-1 expression in db/db retina

Pancreas

WM RT+ RT-

Neuroretina

RT+ RT- Blank

GLP-1Receptor
GLP-1R in human retina

![Image of protein expression and bar graph showing GLP-1R expression in different tissues and conditions.]

- **Protein Expression:**
  - GLP-1R and β-actin bands are shown for different tissue samples (L, RPE, N) across lanes (1-6).
  - GLP-1R bands are noted at 50 kDa.

- **Bar Graph:**
  - Comparing GLP-1R R.Q. across different conditions (LIVER, BOWEL, RPE, NR) for CONTROL and DIABETIC donors.

- **Immunohistochemistry:**
  - Images showing GLP-1R expression in control and diabetic donors, with sections labeled as RPE, PR, ONL, INL, GCL.
Topical administration of GLP-1 agonist prevents diabetes-induced retinal neurodegeneration

Experimental design

- db/+ (non-diabetic control)  
  - n=12

- db/db  
  - GLP-1 eye drops * (400 µg/Kg/d)  
  - Vehicle  
  - n=12

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 days

* liraglutide
GLIAL ACTIVATION

% positive GFAP labeling

<table>
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<th>score</th>
<th>db/+</th>
<th>db/db Vehicle</th>
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*Diabetologia 2014 (Abstract EASD)*
APOPTOSIS

db/+ (control)  db/db + vehicle  db/db + GLP-1 analogue
Glutamate concentration

µmol/g protein

*  
db/+ (control)  db/db + vehicle  db/db + GLP-1 analogue

GLAST immunofluorescence

db/+ (control)  db/db + vehicle  db/db + GLP-1 analogue
CONCLUSIONS

- Topical administration of a GLP-1 agonists prevents retinal neurodegeneration induced by diabetes. This effect can not be attributed to the improvement of blood glucose levels.

- A significant reduction in glutamate-induced excitotoxicity is among the mechanisms by which GLP-1R agonists exerts its beneficial actions.
NEW THERAPEUTIC APPROACH OF DR

Topical administration of GLP-1 agonists

- Targeting prevention by means of a non-invasive method
- Effective in experimental models
- Absence of systemic adverse effects
- No current competitors
- Preclinical → Phase I-IIa → Pase III

Tight control of glycemia and blood pressure

No DR

NPDR

DME-CSDME

PDR

Neurodegeneration as a new pathogenic factor

- Laser photocoagulation
- Intravitreal injections of corticosteroids or anti-VEGF
- Vitreo-retinal surgery
Thank you for your attention!