The role of cannabinoid receptors in renal diseases

Pr. Hélène FRANCOIS

UMR 1197 Interactions cellules souches-niches: physiologie, tumeurs et réparation tissulaire
Renal fibrosis: easier to prevent than to treat.....

Normal kidney

Terminal CKD

Need for novel targets
Unilateral ureteral obstruction model of fibrogenesis

- **Unilateral ureteral obstruction model (OUU) : advantages**
  - Reproducible
  - Rapid developpement of interstital fibrosis

- **Mechanisms involved**
  - Inflammation interstitielle, sécrétion de cytokines et chimioxines
  - Dilatation et atrophie tubulaire
  - Prolifération des myofibroblastes
  - Apoptose
New targets in renal fibrosis

- Cnr1 encoding for the CB1 receptor gene was among the most upregulated genes in UUO
- Results confirmed by RT-qPCR and WB

**Hypothesis:** is CB1 a new target in renal disease?
The cannabinoid system and its receptors

- 2 types of GPCR receptors: CB1 and CB2

- Expression and physiological functions

<table>
<thead>
<tr>
<th>CB1</th>
<th>CB2</th>
</tr>
</thead>
<tbody>
<tr>
<td>• CNS → learning, memory</td>
<td>• Immune cells: T, B, NK-cells, macrophages → anti-inflammatory role</td>
</tr>
<tr>
<td>• Adipose tissue</td>
<td>• Lymphoid tissue: thymus, spleen, bone marrow</td>
</tr>
<tr>
<td>• Intestin → protection</td>
<td>• Liver</td>
</tr>
<tr>
<td>• Liver → protection</td>
<td>• Pancreas → regulation of insulin secretion</td>
</tr>
<tr>
<td>• Endothelium → renal hemodynamics and GFR regulation</td>
<td></td>
</tr>
<tr>
<td>• Uterus/testicle</td>
<td></td>
</tr>
<tr>
<td>• Skin</td>
<td></td>
</tr>
<tr>
<td>➤ metabolic syndrome++</td>
<td></td>
</tr>
<tr>
<td>NEED for PERIPHERAL CB1 blockers</td>
<td></td>
</tr>
</tbody>
</table>
The CB receptor ligands

2 main endogenous ligands (eicosanoids), very short half-life

- **Anandamide (AEA)**
  - higher affinity for CB1 (Ki ≈ 61-543nM) than for CB2 (Ki ≈ 279 - 1940 nM)
  - vasodilation of afferent arterioles
  - reduced GFR
  - also ligand for TRPV1

- **2-arachidonoylglycerol (2-AG)**
  - higher affinity for CB1 (Ki ≈ 58-572nM) than for CB2 (Ki ≈ 145 - 1400nM)
  - Concentrations 800 time higher than ANA
  - inflammation, proliferation, apoptosis
  - metabolism regulation

**2-AG 800 times more abundant** than AEA in most tissues including the kidney
Endogenous cannabinoids during UUO

- Regulation of endocannabinoid synthesis pathways during UUO

Important pathway during renal diseases?
The CB1 blocker rimonabant

- Decrease in body weight, improvement of lipid profile, decrease in microalbuminuria
- Withdrawal from the market in 2008: central nervous system side effects.

The CB1 blocker AM6545

- Peripherally restricted
- Similar efficacy in metabolic syndrome
Cannabinoid receptors and fibrosis in other organs?

Liver
- CB2 antagonism
- CB1 antagonism

Teixeira Clerc et al., 2006
Julien et al., 2005

Heart
- CB1 antagonism
- CB2 agonism

Slavic et al., 2013
Rajesh et al., 2012
Defer et al., 2009

Skin/Lungs
- CB2 antagonism
- CB1 knockout

Marquart et al., 2010
Servettaz et al., 2010

Liver fibrosis
Cardiac fibrosis
Ischemic cardiac fibrosis
Skin and lung fibrosis (systemic sclerosis)
1. Role in normal renal physiology?

2. Role during renal disease during diabetes and metabolic syndrome

3. Other renal disease? Renal fibrosis?
Renal expression of the CB receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Localization (general)</th>
<th>Localization (specific)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB₁</td>
<td>Tissue</td>
<td>Whole kidney</td>
<td>Human, rat, mouse</td>
</tr>
<tr>
<td></td>
<td>Region</td>
<td>Arterioles</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glomerulus</td>
<td>Rat, mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tubules</td>
<td>Human, rat, mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loop of Henle</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collecting ducts</td>
<td>Human</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interstitium</td>
<td>Human, rat</td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td>Podocytes</td>
<td>Rat, mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesangial cells</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proximal tubular epithelial cells</td>
<td>Human, rat, mouse, pig</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Distal tubular epithelial cells</td>
<td>Human, mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intercalated cells</td>
<td>Human</td>
</tr>
<tr>
<td>CB₂</td>
<td>Tissue</td>
<td>Whole kidney</td>
<td>Rat</td>
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<td></td>
<td></td>
<td>Mesangial cells</td>
<td>Rat</td>
</tr>
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</table>
CB1 expression during UUO

- no CB1 expression in Cnr1⁻/⁻ mice
- Increased CB1 expression in UUO

CB1 expressed in the:
  - interstitium
  - glomeruli
  - tubules

*Lecru et al Kidney Int 2015*
CB2 expression during UUO

- Cnr2 expression by RT-qPCR

- CB2 expression in the kidney

  - no CB2 expression in normal kidneys
  - Increased CB2 expression in UUO
  - CB2 is expressed in the
    - interstitium
    - less so in glomeruli and tubules

Lecru et al Kidney Int 2015
Expression du récepteur CB1 dans les néphropathies

- CB1 expression by IHC, morphometric quantification

 Increased CB1 expression in:
- IgA nephropathy
- Acute interstitial nephritis
- Diabetic nephropathy

Lecru et al. Kidney Int 2015
1. Regulation of renal hemodynamics and GFR

**Vascular function**
- AEA injection leads to Afferent arteriole et Efferent arteriole vasodilation through NO synthesis\(^1\)
- Preferential vasodilation of the efferent arteriole: decrease in GFR through CB1 receptor\(^2\)
- AEA injection induces a fall in blood pressure\(^1-3\)

1 Deutsch et al JCI 1997, 2 Koura JASN 2004, 3 Li et al J Hypertension 2006

**Tubular function??**
- AEA injection increases diuresis but not natriuresis\(^1,2\)
- AEA increases natriuresis through CB1 action in the loop of Henle\(^3,4\)

The cannabinoid system in renal disease?

1. Role in normal renal physiology?

2. Role during renal disease during diabetes and metabolic syndrome

3. Other renal disease? Renal fibrosis?
CB1 in diabetic nephropathy and metabolic syndrome

- Decrease in body weight, insulin resistance, blood glucose

Model of Zucker rat and diabetic mouse db/db

- CB1 antagonism (rimonabant, AM6545 and AM251, JD 5037)
  - → decrease in serum creatinine
  - → reduction of tubulo-interstitial lesions
  - → decreased albuminuria

Zucker rat
- Janiak et al., 2007
- Jourdan et al., 2014

db/db mice
- Barutta et al., 2010
- Nam et al., 2012

- → reduction of mesangial expansion
- → reduction of Coll IV accumulation in the mesangium
- → decreased serum creatinine
- → decreased albuminuria
CB1 in diabetes: physiopathology?

Zucker rat

Db/db mice

- better expression of slit diaphragm proteins
- hemodynamic effects in glomeruli?
  - Through a decrease in oxidative stress
- Increased megalin expression in proximal tubules and thus decreased albuminuria

Barutta et al., 2010
Nam et al 2012

Role in overt diabetic nephropathy?
Role in renal fibrosis?

Direct effect in non metabolic renal disease?

Jourdan et al 2014
CB2 in diabetic nephropathy and metabolic syndrome

- Same BP, Blood glucose level, body weight in CB2 knockout mice

Diabetes induced by streptozotocin

- CB2 agonist

  → increased nephrin and ZO-1
  → decreased monocyte infiltration
  → decreased albuminuria

Streptozotocin
Barutta et al 2011

CB2 knockout
Streptozotocin
Barutta et al 2014

  → increased mesangial expansion
  → decreased serum creatinine
  → increased albuminuria
  → increased Coll I accumulation in the mesangium

Direct role of CB2 on the podocyte
The cannabinoid system in renal disease?

1. Role in normal renal physiology?

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The myofibroblast is the main effector cell in fibrosis

- synthesizes extra-cellular matrix proteins
  - Fibronectin
  - Proteoglycans
  - Type I and III collagens

- main characteristics
  - Proliferation, contraction
  - Morphology: microfilaments, well developed ER, high expression of adhesion molecules
  - Phenotype: α-SMA, S100A4, vimentin expression
Various sources of myofibroblasts

d’après Lecru et al.
Increased 2-AG synthesis in obstructed kidneys versus controls

Renal 2-AG levels > brains 2-AG levels

Role during UUO?
- **Cnr1 knockout**: CD1 outbred background Cnr1\(^{-/-}\) and matched Cnr1\(^{+/+}\) (Ledent et al., 1999)

- **Pharmacologic model**: C57BL/6 inbred mice treated with selective antagonists

  - Daily gavage
  - Sacrifice
  - CB1 antagonist: Rimonabant
  - CB2 antagonist: SR144528
  - More fibrosis?
CB1 inhibition decreases renal fibrosis in UUO

- **Cnr1 knockout**
  - Decreased fibrosis in cnr1 knockout mice, $n=6-11, p<0.05$

- **Pharmacological blockade**
  - Decreased fibrosis (rimonabant and AM6545), $n=7, p<0.05$
CB1 inhibition decreases fibrosis

- RT-qPCR and WB

**Decreased expression in Col1a2 and Col3a1 in obstructed kidneys in Cnr1^{-/-} and rimonabant treated mice**
Role of CB2 in UUO

Since CB1 and CB2 have opposite roles in liver fibrosis
→ **Hypothesis**: potentialization of CB1 anti-fibrotic role?

- CB2 antagonist: **SR144528**?
- CB2 agonist: **JWH133**?
- combined effect with CB1 blockade?

- aggravation of renal fibrosis for SR144528
  - decreased fibrosis for JWH133
  - no additive effect with rimonabant

**Prominent role of CB1 in renal fibrosis during UUO**
CB1 and CB2 role in renal fibrosis

Endocannabinoids

Myofibroblasts

Collagens
CB1 expressing cells?

- No CB1 expression on T cells and macrophages
- Co-expression of CB1 and α-SMA in the interstitium
- CB1 expression on myofibroblasts.
**TGF-β1 expression**

- Increased renal TGF-β1 with the progression of CKD
- Myofibroblasts activation → increased collagen synthesis

No change in TGF-β1 expression of target genes by CB1 blockade
CB1 blockade does not modify myofibroblast number

- **No difference in myofibroblast markers in Cnr1⁻/⁻ and rimonabant treated mice**

- **No change in myofibroblasts number**
Role of CB1 on myofibroblasts in vitro

- CB1 expression on myofibroblasts in vitro
  - Increased CB1 expression by TGF-β1 on myofibroblasts

- AEA synthesis by myofibroblasts in vitro

  - Autocrine/paracrine regulation of AEA
Role of CB1 blockade on myofibroblasts *in vitro*

- **Increased expression of collagène III by myofibroblasts *in vitro***

- **Col3a1**

  - Basal myofibroblasts + DMSO
  - Myofibroblasts + TGFβ1 5ng/ml 1h + DMSO
  - Myofibroblasts + TGFβ1 5ng/ml 1h + rimonabant 1µM

- **Direct effect of CB1 on myofibroblasts**

- Decreased Col3a1 transcription in rimonabant treated myofibroblasts and in Cnr1−/− myofibroblasts.

- **Direct effect of CB1 on myofibroblasts.**
Effet du blocage de CB1 les myofibroblastes *in vitro*

- Increased MCP-1 synthesis through TGF-β1 activation
- Decreased MCP-1 synthesis by rimonabant *in vitro*
Rimonabant effect on macrophage infiltration in UUO

- **Role of rimonabant**

  - No effect on T-cell recruitment
  - Decreased macrophage infiltration with rimonabant and not with AM6545

- **Macrophages characteristics in UUO**

  - Decreased M1-type macrophages
  - CB1 independent pathway? TRPV1?

  ![Macrophages images](image_url)

  ![Graphs showing CD3 and F4/80 infiltration](image_url)

  ![Graphs showing iNOS, IL23, MCP1, Arg1, and Mrc1 expression](image_url)
rimonabant and AM6545 pathways

Rimonabant and AM6545 pathways involve anti-fibrotic and anti-inflammatory roles. Other cell types and receptors, such as TRPV1, are also implicated in these pathways. Endocannabinoids play a role in autocrine-paracrine regulation. Myofibroblasts are targeted by these pathways.

Collagens are also shown to be affected by these pathways.
Conclusion

In UUO and various nephropathies

- CB1 expression is increased in various human nephropathies.
- CB1 and CB2 expression as well as their endogenous ligands is increased during UUO.
- CB1 blockade reduces renal fibrosis in UUO.
- CB2 agonists do not potentialize CB1 blockade in UUO.
- CB1 blockade action directly involves the myofibroblasts in renal fibrosis during UUO.

(Lecru et al Kidney Int 2015)

During obesity and early stages of diabetic nephropathy

- CB1 blockade decreases insulin resistance, albuminuria and improves renal function in Zucker rat and in db/db mice
- Improvement of podocyte function, decreased oxydative stress markers and fibrosis. (Barutta et al 2011, 2014)
Conclusion

CB1 blockers: a new therapeutic target in renal diseases and CKD?

• Direct role on the podocyte and the myofibroblast
• Good cardiovascular safety profile and SNS profile (peripherally restricted blockers)
Lola LECRU
   Antoine DURRBACH
   Aimé VAZQUEZ
   Bernard CHARPENTIER
   Julien GIRON-MICHEL
   Bruno AZZARONE
   Hans LORENZO
   Pierre EID
   Séverine LECOURT

Sophie FERLICOT
   Aurore DEVOCELLE
   Amélie VERNOCCHET
   Cathy ALEXIA
   Séverine BEAUDREUIL

Etudiants
   Meriem DALIA
   Djenaba BA
   Myriam SAID
   Myriam DAO

Plateforme d’animalerie de l’IAL
   Ibrahim CASAL
   Mélanie POINT et Xavier BIOLCHINI

INSERM 972
   Christophe DESTERKE (microarray)

Plateforme de Spectrométrie de Masse MasSpecLab
   Stanislas GRASSIN-DELYLE

INSERM 702
   Christos CHATZIANTONIOU
   Sophie VANDERMEERSCH