Updates in Peritoneal Membrane pathophysiology

Eric Goffin
Cacéres 2016
EURO 2016 BETTING ODDS

The 2016 European Football Championship will take place in France.

**EURO 2016 OUTRIGHTS**

<table>
<thead>
<tr>
<th>Winner</th>
<th>Odds</th>
<th>Bookmaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>17/5</td>
<td>bwin.com</td>
</tr>
<tr>
<td>France</td>
<td>7/2</td>
<td>betfair</td>
</tr>
<tr>
<td>Spain</td>
<td>11/2</td>
<td>totesport</td>
</tr>
<tr>
<td>Belgium</td>
<td>12</td>
<td>Coral</td>
</tr>
<tr>
<td>England</td>
<td>12</td>
<td>bwin.com</td>
</tr>
</tbody>
</table>

**Other Outright Betting**

- **To Qualify**
  - England, Northern Ireland

- **Top Goalscorer**
  - Thomas Muller (8), Cristi

- **Winner/Top Goalscorer**
  - Germany/Thomas Muller
Identification, Purification, and Partial Characterization of a Novel Mr 28,000 Integral Membrane Protein from Erythrocytes and renal Tubules

Denker BM, Smith BL, Kuhajda FP, Agre P.

Appearance of Water Channels in *Xenopus* Oocytes Expressing Red Cell CHIP28 Protein

Gregory M. Preston, Tiziana Piazza Carroll, William B. Guggino, Peter Agre*
Aquaporin-1

- Structure: channel of 3.0 Å
- Specific to water (not to glucose or urea)
- Discovered in red cells
- 3,000,000,000 molecules H₂O/second
Physiological background

Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
Physiological background

Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
Computer Simulations: the 3 pores model

**Capillary**
- Small pores: 40-50 Å
  - Transport of solutes
  - Transport of water

**Interstitium**
- Large pores: 150-300 Å, 3% of the surface
  - Protein losses

**Mesothelium**
- Ultrasmall pores: <3 Å
  - Free water transport

Rippe et al, Kidney Int 1991
Johann Morelle, by courtesy
Transport of sodium and water: ultrasmall and small pores

Ultrasmall pores
50% of UF
Transport of free water
Sodium sieving
Transport of sodium and water: ultrasmall and small pores

Ultrasmall Pores
- 50% of UF
- Transport of free water
- Sodium sieving

Small pores
- 50% of UF
- Transport of water + sodium
  - Convection
  - Diffusion
AQP1 is expressed within the peritoneal capillaries
Role of AQP1 in the transport of water in PD

Mechanisms of water transport

- Wild-type mice, $Aqp1^{+/+}$
  - Small pores
  - AQP1

- Knock-out mice, $Aqp1^{-/-}$
  - Small pores
AQP-1 is the molecular counterpart of the ultrasmall pore

\[ \text{D/P Sodium} \]

<table>
<thead>
<tr>
<th>Dwell Time (min)</th>
<th>AQP1 (+/+) (n=6)</th>
<th>AQP1 (+/-) (n=6)</th>
<th>AQP1 (-/-) (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.75 ± 0.03</td>
<td>0.80 ± 0.02</td>
<td>0.85 ± 0.04</td>
</tr>
<tr>
<td>30</td>
<td>0.89 ± 0.04</td>
<td>0.97 ± 0.03</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>60</td>
<td>0.94 ± 0.05</td>
<td>0.97 ± 0.04</td>
<td>0.95 ± 0.06</td>
</tr>
<tr>
<td>90</td>
<td>0.98 ± 0.06</td>
<td>0.97 ± 0.05</td>
<td>0.97 ± 0.07</td>
</tr>
<tr>
<td>120</td>
<td>1.00 ± 0.07</td>
<td>0.97 ± 0.06</td>
<td>0.99 ± 0.08</td>
</tr>
</tbody>
</table>

*# Significant differences compared to control group.

\( Aqp1 (+/+) (n=6) \)
\( Aqp1 (+/-) (n=6) \)
\( Aqp1 (-/-) (n=6) \)

It is now widely accepted that AQP-1 is the molecular counterpart of the ultrasmall pore predicted by computer simulations of water transport in Peritoneal Dialysis patients.
Physiological background

Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
From mice to men ... free water transport in PD

Amsterdam data – SPA test
60 min – hypertonic dwell
Incident PD patients, n=211

Great inter-individual variability → genetic factors?
rs2075574 variant within *Aqp1 gene promoter* influences free water transport in PD patients

**Cohort of 211 patients**

<table>
<thead>
<tr>
<th>rs2075574</th>
<th>CC n = 94</th>
<th>CT n = 94</th>
<th>TT n = 23 (11%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport Parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UF (DS)</td>
<td>588 (338)</td>
<td>673 (309)</td>
<td>483 (266)</td>
<td>0.02</td>
</tr>
<tr>
<td>Na sieving (DS) (n = 155)</td>
<td>0.05 (0.03)</td>
<td>0.05 (0.02)</td>
<td>0.04 (0.03)</td>
<td>0.046</td>
</tr>
<tr>
<td>D/P_{creat} 4h (DS)</td>
<td>0.72 (0.12)</td>
<td>0.73 (0.11)</td>
<td>0.76 (0.12)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Maréchal C et al. ASN 2010*
Promoter Variant of \textit{AQP1}: Influence on Survival

\textbf{UCL – KUL} \hspace{1cm} (n=187)

\textbf{NECOSAD} \hspace{1cm} (n=459)

Maréchal C et al. ASN 2010
Immunostaining for AQP1 in Peritoneal Biopsies

Patient – rs2075574 CC

Patient – rs2075574 TT
A genetic heterogeneity within the AQP1 gene could explain some untoward evolutions in PD patients.
Physiological background

Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
Dexamethasone accelerates fetal lung maturation
Corticosteroids increase AQP1 expression and free water transport within the rat peritoneal membrane

Transport parameters before and after renal transplantation

A

Sodium sieving (%)

USP Ultrafiltration (mL)

D/P creatinine

Before
After

Before
After

Before
After

de Arteaga J et al Nephrol Dial Transplant 2011; 26: 4142-4145
Changes in osmotic water transport through regulation of AQP1 activity

Plants have the ability to modulate AQP-mediated osmotic water transport in response to environmental conditions.

Courtesy of Olivier Devuyst
Changes in osmotic water transport through regulation of AQP1 activity

Intracytoplasmic D loop is involved in the regulation of AQP-mediated water transport in plants (*gating*).

*Tournaire-Roux et al, Nature 2003*
*Chaumont et al, Biol Cell 2005*
*Tornroth-Horsefield et al, Nature 2006*

*Courtesy of Olivier Devuyst*
AqF026, a loop diuretic derivative, is an agonist of AQP-1 that can increase water transport *in vitro* → *in vivo* efficacy?
AqF026, the first identified agonist of AQP1

**In vitro and in silico**

- **AqF026**
- **Aqp1** untreated

**Relative increase in net UF**

- **Aqp1** untreated (11)
- AQP1 untr (12)
- AQP1 026 (12)
- cont untr (13)
- cont 026 (13)

**Dwell time (min)**

- 0
- 30
- 60
- 90
- 120

**IP volume (ml)**

- **AqF026**
- **DMSO**

**p = 0.016 between curves**

**3.86% glucose**

**In vivo**

- **AqF026**
- **Aqp1** untreated

**Relative increase in net UF**

- **Aqp1** untreated

**AqF026 (µM)**

- 0
- 0.75
- 1.5
- 7.5
- 15
- 30

**p = 0.016 between curves**
A pharmacological modulation of AQP1 is possible with corticosteroids administration, or by using AQP1 agonists; Studies in PD patients?
Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
Peritonitis presentation: the classical three signs

Cloudy effluent
Abdominal pain
WCC > 100 Leucocytes/uL of dialysate, > 50 % PMN

Decrease in UF

Positive culture in approx 80 %
Importance of an early diagnosis and appropriate therapy

Consequences of peritonitis?

IMMEDIATE
hospitalisation
HD transfer
mortality

LONG TERM
cardiovascular
mortality

Mujais S Kidney Int 2006
Johnson DW et al Am J Kidney Dis 2009
Acute peritonitis: structural changes

Rat model - Catheter-induced peritonitis - 5 days

Control

Peritonitis

Inflammatory infiltrate

Control

Peritonitis

Vascular proliferation

Combet S et al JASN 10: 2185-96, 1999
Peritonitis induce functional and structural peritoneal membrane alterations

Davies S et al Nephrol Dial Transplant 1996
Williams J et al Kidney Int 2003
Role of AQP1 in vascular proliferation during peritonitis?
Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption.

Saadoun S, Papadopoulou MC, Hara-Chikuma M, Verkman AS.

Motile AQP1-expressing cells had prominent membrane ruffles at the leading edge with polarization of AQP1 protein to lamellipodia, where rapid water fluxes occur.

... fundamental role of water channels in cell migration, which is central to angiogenesis, wound healing, and organ regeneration.
Histological examination of tumours - AQP1 staining

AQP1+/+

AQP1-/-

200 μm

- Aqp1 KO: fewer microvessels
- islands of necrotic areas in tumours

AQP1 deletion impairs tumour microvessels proliferation → necrosis
Peritonitis induce functional and structural peritoneal membrane alterations

Aqp1 gene deletion reduces vascular proliferation during peritonitis

Aqp1 gene deletion prevents loss of peritoneal function during peritonitis

Clinical perspectives of AQP 1 targeting in acute peritonitis?
Physiological background

Clinical variability

Regulation of AQP-1 activity?

Acute peritonitis

Long-term PD patients
Long-term exposure to non-physiologic PD solutions alters the peritoneal membrane.
Lifestyle Measures Recommended for Patients with Osteopenia

- Peritonitis
- Hemoperitoneum
- PD Fluids:
  - Glucose (AGEs, GDPs)
  - Lactate, low pH

Adapted from Lopez-Cabrea et al. (2006)
Long-term exposure to non-physiologic PD solutions alters the peritoneal membrane

**Structural alterations**

**Functional alterations**

- **Vascular proliferation**
- **Faster glucose absorption from the dialysate**
- **Loss of UF capacity**
  - (<400 ml/4h with a 3.86% PET)

*up to 50% of PD patients after 6 years*
Functional changes in peritoneal transport during long-term PD

Longitudinal follow-up of 574 incident PD patients
Stoke-on-Tent (UK) cohort

Progressive increase in solute transport and loss of UF capacity

Davies, J Am Soc Nephrol 2004
UF failure: AQP dysfunction?

→ Structure: narrow pore 3.0 Å
→ Specificity for water only (no urea, glucose)
→ Distribution in endothelium
Expression of Aquaporin-1 in a Long-Term Peritoneal Dialysis Patient With Impaired Transcellular Water Transport

Eric Goffin, MD, Sophie Combet, MS, François Jamar, MD, PhD, Jean-Pierre Cosyns, MD, and Olivier Devuyst, MD, PhD

- May 1997: the patient died from pulmonary sepsis
- Post-mortem analysis of the peritoneum was performed

**Loss of sodium sieving with normal AQP1 expression → abnormal AQP1 structure/function?**

Goffin E et al Am J Kidney Dis 1999
Encapsulating peritoneal sclerosis (EPS)

- Rare complication of PD (overall incidence ranging 0.5-3.3%)
- In the majority of patients, after PD treatment has stopped
- Dominant risk factor for EPS: cumulative duration of exposure to PD fluids
- 50% mortality rate (recurrent bowel occlusion, malnutrition, infections)
- Unelucidated pathophysiology
- No definitive criteria enable detection of the early stages of EPS, although this condition is frequently preceded by loss of UF capacity

Korte et al, Nat Rev Nephrol 2010;
Patients who develop EPS have a premature loss in UF capacity

Case-control study
Stoke-on-Tent cohort, UK
Yearly PET 2.27% glucose

- Excessive loss in ultrafiltration capacity in patients who will develop EPS
- Cannot be fully explained by a faster transport of small solutes

Lamie et al, Kidney Int 2010
Evolution of peritoneal transport parameters (within PD – before EPS occurrence)

Early loss of UF and Na sieving in patients in whom EPS will develop
**Multivariate analysis**

Loss of sodium sieving in long-term PD patients = *strong independent indicator of EPS occurrence*

Patients who will develop EPS exhibit an early loss of free water transport (net UF and sodium sieving)

→ Alteration of AQP-1 water channels?
AQP1 peritoneal expression in long-term vs EPS patients

No modification of AQP1 vascular density in EPS vs control patients

Morelle...Devuyst, Goffin J Am Soc Nephrol 2015
Patients who will develop EPS exhibit an early loss of free water transport (net UF and sodium sieving)

→ *Effect of fibrosis?*
Structural modifications of the peritoneal structure in EPS patients

Collagen fibers density and properties

<table>
<thead>
<tr>
<th>Densité fibres collagène</th>
<th>Lumière polarisée</th>
<th>Lumière polarisée</th>
<th>Lumière polarisée</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urémie</td>
<td>DP au long cours</td>
<td>PSE</td>
<td>DP au long cours</td>
</tr>
</tbody>
</table>

Diamètre des fibres
- > 60 nm
- 20-40 nm

Collagène
- Type I
- Type III

Processus de réparation tissulaire
- Stades avancés
- Stades précoces

Collagen volume fraction (% submesothelial area)
Fibrosis and water transport across the peritoneal membrane

The thicker and the more dense in collagen fibers,

The lower the peritoneal membrane is permeable to water

Vascular and fibrotic changes in the EPS peritoneum

Morelle...Devuyst, Goffin, J Am Soc Nephrol 2015
The ultrafiltration capacity and the sodium sieving are progressively reduced when the vascular area and the interstitial fibrosis both progress in parallel despite the fact that the capillary $\alpha_c$ (rel. capillary density of AQP1) is kept unchanged.
Expression of Aquaporin-1 in a Long-Term Peritoneal Dialysis Patient With Impaired Transcellular Water Transport

Eric Goffin, MD, Sophie Combet, MS, François Jamar, MD, PhD, Jean-Pierre Cosyns, MD, and Olivier Devuyst, MD, PhD

Clinical follow-up:

- Two episodes of intestinal subocclusion (April and May 1996), with no identified etiology
- Progressive weight loss, inflammatory syndrome, food intolerance and total parenteral nutrition
- Abdominal CT (02/1997): «calcified thickening of the intestinal wall with anterior parietal calcifications»
- The patient died of pulmonary sepsis
- Autopsy showed a «thickened, tanned, brown peritoneum with a leathery appearance»

Retrospective diagnosis of EPS?
Expression of Aquaporin-1 in a Long-Term Peritoneal Dialysis Patient With Impaired Transcellular Water Transport

Eric Goffin, MD, Sophie Combet, MS, François Jamar, MD, PhD, Jean-Pierre Cosyns, MD, and Olivier Devuyst, MD, PhD

67 yo patient, on PD for 11 years
UF failure and loss of sodium sieving

Retrospective diagnosis of EPS and severe peritoneal fibrosis as the cause of sodium sieving and osmotic conductance in this patient?

Morelle J et al Perit Dial Int in press
Interstitital fibrosis restricts osmotic water transport

This functional change is linked to specific alterations of the collagen matrix in the peritoneal membrane of patients with EPS, with

- excessive fibrosis
- increased collagen density
- greater proportion of thick collagen fibers

Alteration of AQP 1 permeability ?
1. Crucial role of AQP1 in PD patients to promote adequate ultrafiltration

2. Pharmacological regulation of AQP1 in PD patients seems plausible using agonists … rather than steroids

3. Deleterious role of AQP1 in the context of acute peritoneal inflammation related to peritonitis

4. Peritoneal membrane alteration in the long-term is linked to specific alterations of the collagen matrix ….