Aristolochic acid nephropathy: *from bed to benchside*

F. Debelle, JL. Vanherweghem, J. Nortier

Erasme university hospital
Experimental nephrology unit
Université Libre de Bruxelles
Revisiting AAN…
Revisiting AAN…

1. The Belgian outbreak of « Chinese-herb nephropathy »
2. A worldwide problem
3. Clinical and experimental toxicity of AA
4. Perspectives of research
1. « Chinese-herb nephropathy »

<table>
<thead>
<tr>
<th>Month</th>
<th>Case n°1</th>
<th>Case n°2</th>
<th>Case n°3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>Creat. 1.1*</td>
<td></td>
<td>Creat. 0.8*</td>
</tr>
<tr>
<td>May 90</td>
<td></td>
<td></td>
<td></td>
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<td>March 91</td>
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<td>Nov 91</td>
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<td></td>
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<tr>
<td>Jan 92</td>
<td>Creat. 3.7*</td>
<td>Creat. 3.8*</td>
<td>Creat. 3.0*</td>
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<td>March 92</td>
<td><em>Renal Biopsy</em>*</td>
<td><em>Renal Biopsy</em>*</td>
<td><em>Renal biopsy</em>*</td>
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<tr>
<td>Dialysis</td>
<td>April 92</td>
<td>June 92</td>
<td>July 92</td>
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</tbody>
</table>

* mg/dl
** Renal biopsy: interstitial fibrosis

Exposure to Chinese herbs
Vanherweghem JL et al.

Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs


Courtesy Dr Baleriaux
### Composition of slimming pills prescribed before 1990 and from May 1990 to 1992

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td><strong>Intradermal injections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artichoke extracts</td>
<td>0.2</td>
<td>0.2 *</td>
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<tr>
<td>Euphyllin</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td><strong>Tablets A</strong></td>
<td></td>
<td></td>
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<tr>
<td>Fenfluramine</td>
<td>17-25</td>
<td>17-25</td>
</tr>
<tr>
<td>Diethylpropion</td>
<td>17-25</td>
<td>17-25</td>
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<tr>
<td>Meprobamate</td>
<td>0-5</td>
<td>0-5</td>
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<tr>
<td><strong>Tablets B</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pancreas powder</td>
<td>100</td>
<td>0</td>
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<tr>
<td>Laminaria powder</td>
<td>50</td>
<td>0</td>
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<tr>
<td>Fucus extracts</td>
<td>50</td>
<td>0</td>
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<tr>
<td>Cascara powder</td>
<td>20-150</td>
<td>20-150</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>25-45</td>
<td>25-45</td>
</tr>
<tr>
<td>Belladona extracts</td>
<td>0</td>
<td>1-2</td>
</tr>
<tr>
<td><strong>Stephania tetrandra</strong></td>
<td>0</td>
<td>100-200</td>
</tr>
<tr>
<td><strong>Magnolia officinalis</strong></td>
<td>0</td>
<td>100-200</td>
</tr>
</tbody>
</table>

*a From Vanherweghem et al. [Lancet 1993]

* stopped on July 1991

$ Orally ingested, 3 times per day
«Stephania Tetrandra»

Han Fang-Ji

«Stephania Tetrandra»

Han Fang-Ji

«Stephania Tetrandra»

Han Fang-Ji

©Stephania Tetranda©
Han Fang-Ji

Aristolochic acid I + II
(R=OCH₃ / H)


Aristolochic acid I + II
(R=OCH₃ / H)

From Qian (1996)
1, twig leaf; 2, longitudinal section; 3, fruit twig; 4, flower
Aristolochia fangchi

Aristolochic acid I + II
(R=OCH₃ / H)

From Qian (1996)
1, twig leaf; 2, longitudinal section; 3, fruit twig; 4, flower

Aristolochia fangchi

Aristolochic acid I + II
(R=OCH₃ / H)


From Qian (1996)
1, twig leaf; 2, longitudinal section; 3, fruit twig; 4, flower
Potential toxicity of herbal and plant products

Huxtable RJ. *Drug Safety* (1990)
Potential toxicity of herbal and plant products

1. Correct identification … but unknown or underestimated toxicity
Potential toxicity of herbal and plant products

1. Correct identification … but unknown or underestimated toxicity

2. Accidental contamination or deliberately modified composition

Huxtable RJ. *Drug Safety* (1990)
Potential toxicity of herbal and plant products

1. Correct identification … but unknown or underestimated toxicity

2. Accidental contamination or deliberately modified composition

3. Misidentification of the plant or substitution by another more toxic compound

Huxtable RJ. *Drug Safety* (1990)
Table 4. Relationship between the cumulative doses of *ST–AF* and the risk of developing ESRD

<table>
<thead>
<tr>
<th>Cumulative dose of <em>ST–AF</em> (g)</th>
<th>No. of patients with CRF</th>
<th>No. of patients with ESRD</th>
<th>Total No. of patients</th>
<th>Risk of ESRD (%)</th>
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</thead>
<tbody>
<tr>
<td>0–99</td>
<td>9</td>
<td>4</td>
<td>13</td>
<td>30.8</td>
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<tr>
<td>100–199</td>
<td>13</td>
<td>24</td>
<td>37</td>
<td>64.9</td>
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<tr>
<td>200–299</td>
<td>3</td>
<td>9</td>
<td>12</td>
<td>75.0</td>
</tr>
<tr>
<td>300–399</td>
<td>2</td>
<td>7</td>
<td>9</td>
<td>77.8</td>
</tr>
</tbody>
</table>

*ST–AF*, *Stephania tetrandra* replaced by *Aristolochia fangchi*.
End-stage CHN: evolution of renal replacement therapies

Follow-up (years)

Patients AAN en IRT

- Dialysés
- Transplantés
- Décédés
- Nb cumulatif de patients

Années de suivi
Follow-up (years)
Autoradiogram of specific AA-related DNA adducts in renal tissue


Arlt VM. Mutagenesis (2002)
Autoradiogram of specific AA-related DNA adducts in renal tissue


Arlt VM. Mutagenesis (2002)
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The Chenonceau castle

The green cabinet, Catherine de Médicis’s study
The Brussels « Aristolochia Tapestry »
(15th century)
Complementary and Alternative Medical (CAM) Therapies

- Acupuncture
- Relaxation techniques
- Massage
- Reflexology

= alternative to or complementary to «Western» conventional medicine

- Folk remedies
- Herbal medicines
The escalating use of alternative therapies

- **very popular**: 47% of the adult US population (1997), expenditure over $21 billion, < 40% disclosed to physicians, 79% of patients perceived the combination to be superior to either one alone

  Eisenberg DM et al. *NEJM* 1993; *JAMA* 1998; *Arch Int Med* 2001

- « natural » plant origin → safe !!!

- **traditional medicines**: equal official status with Western medicine (Africa, Asia)

- **dietary supplements** (not regulated by the FDA)
  easy available, over-the-counter, low cost
A pharmacy of traditional herbal medicine (Hangzhou, China)

Courtesy Dr F. Debelle
The wide spectrum of indications…

- Eczema
- Liver enhancement, hepatitis B
- Arthritis, rheumatism
- Pain relief
- …etc…

Aristolochia fangchi

From Qian (1996)
1, twig leaf; 2, longitudinal section; 3, fruit twig; 4, flower

Aristolochia manshuriensis

From Qian (1996)
1, fruit twig; 2, flower; 3, transverse section

Guang Fang-Ji

Guan Mutong
FDA recommendations (2001)

Carcinogenicity to humans recognized by the International Agency for Research on Cancer (2002) and the National Toxicology Program (2008)
Aristolochic Acid, an Herbal Carcinogen, Sold on the Web after FDA Alert

TO THE EDITOR: In 2001, the Food and Drug Administration (FDA) issued warnings and an import alert that herbal products are unsafe if they contain or are suspected to contain aristolochic acid.¹ ...

... Despite the actions of the FDA, in 2003 we identified 19 products containing aristolochic acid and 95 products suspected to contain aristolochic acid for sale on the Web. These products and approximately 100 related Web sites are listed on the Web at http://potency.berkeley.edu/aristolochicacid.html. ...

¹ http://www.cfsan.fda.gov/~dms/ds-bot.html

AAN cases around the world

Aristolochia clematidis
Aristolochic acid and the etiology of endemic (Balkan) nephropathy


*Laboratory of Chemical Biology, Department of Pharmacological Sciences, and ²Department of Pathology, Stony Brook University, Stony Brook, NY 11794; ³Department of Urological Surgery and ¹Department of Pathology, Josip Beneš University Hospital, 35000 Slavonski Brod, Croatia; ⁴Roche Molecular Systems, Pleasanton, CA 94588; ⁵Institute Rudjer Bosković, 10000 Zagreb, Croatia; ⁶Division of Environmental Disease Prevention, Wadsworth Center, New York State Department of Health, Albany, NY 12201; ⁷Department of Nephrology and Arterial Hypertension, Zagreb University School of Medicine and University Hospital Center, 10000 Zagreb, Croatia; and §Croatian Center for Endemic Nephropathy, 35000 Slavonski Brod, Croatia

Courtesy of Dr B. Jelakovic
The two faces of Janus?

Chinese herb nephropathy (1992)

Aristolochic acid specific DNA adducts

Aristolochic acid nephropathy (1996 - )

Similarities

Renal interstitial fibrosis and urothelial carcinoma

Balkan endemic nephropathy (1956)

Balkan endemic nephropathy – Aristolochic acid (2007 - )

Similarities

Renal interstitial fibrosis, urothelial carcinoma and AA-related DNA adducts
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Clinical presentation of AAN

Cosyns et al. Kidney Int 1994
The proximal tubule = target of AA

Markers of structural and functional injury:
- brush border enzymuria
- microproteinuria

Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells.
AA-associated urothelial malignancies

### Ureteronephrectomies / AAN patients

<table>
<thead>
<tr>
<th>Center</th>
<th>N. patients</th>
<th>N. urothelial carcinoma</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCL</td>
<td>10</td>
<td>4</td>
<td>Am J Kidney Dis 1999; 33: 1011</td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

**Prevalence +/- 40% !**
<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Pelvis and/or Ureter</th>
<th>Bladder</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>1</td>
<td>T3</td>
<td>High grade pT1</td>
<td>Dead in generalization</td>
</tr>
<tr>
<td>1</td>
<td>T2</td>
<td>pTis</td>
<td>Dead in generalization</td>
</tr>
<tr>
<td>1</td>
<td>T1</td>
<td>pTis</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>1</td>
<td>pTis</td>
<td>High grade pT1</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>3</td>
<td>pTis</td>
<td>pTis</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>1</td>
<td>pTa</td>
<td>High grade pTa + pTis</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>1</td>
<td>pTis</td>
<td>Low grade pTa</td>
<td>Dead from hepatocarcinoma</td>
</tr>
<tr>
<td>2</td>
<td>pTis</td>
<td>Low grade pTa</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>1</td>
<td>pTis + pTa</td>
<td>High grade pTa</td>
<td>Alive with no evidence of disease</td>
</tr>
<tr>
<td>5</td>
<td>pTis</td>
<td>-</td>
<td>Alive with no evidence of disease</td>
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<tr>
<td>1</td>
<td>-</td>
<td>PT3N1</td>
<td>Dead in generalization</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>Low grade pTa</td>
<td>Sudden death</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>Low grade pTa</td>
<td>Alive with no evidence of disease</td>
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Experimental AAN
Experimental AAN

Chronic AA toxicity in rabbits: A model of Chinese herbs nephropathy?

Experimental AAN

Chronic AA toxicity in rabbits: A model of Chinese herbs nephropathy?


Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats

Debelle FD et al. JASN (2002)
Experimental AAN

Chronic AA toxicity in rabbits: A model of Chinese herbs nephropathy?


Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats

_Develle FD et al. JASN (2002)_
Debelle F et al. JASN (2002)
Debelle F et al. JASN (2002)
Debelle F et al. JASN (2002)
Debelle F et al. JASN (2002)

CONTROL

AA 10 mg/kg
Debelle F et al. JASN (2002)

CONTROL

AA 10 mg/kg Day 35
CONTROL

AA 10 mg/kg Day 35

Debelle F et al. JASN (2002)
« The severity of the tubulointerstitial damage determines the functional prognosis »

Schainuck et al. *Hum Pathol* (1970)
« The severity of the tubulointerstitial damage determines the functional prognosis »

Schainuck et al. Hum Pathol (1970)
« The severity of the tubulointerstitial damage determines the functional prognosis »

Schainuck et al. *Hum Pathol* (1970)

« Injury and matrix remodelling predict progression »

Halloran P. *Necker Seminars* (2009)
« The severity of the tubulointerstitial damage determines the functional prognosis »
Schainuck et al. Hum Pathol (1970)

« Injury and matrix remodelling predict progression »
Halloran P. Necker Seminars (2009)
« The severity of the tubulointerstitial damage determines the functional prognosis »

Schainuck et al. *Hum Pathol* (1970)

« Injury and matrix remodelling predict progression »

Halloran P. *Necker Seminars* (2009)

Early events promoting fibrosis?
Experimental design

**AA group** (10 mg of AA/ kg of bw, sc)

**Control group** (AA vehicle, sc)

Acclimatization

Days of sacrifice

1 2 3 4 5 7 10 14 18 35

Acute phase

Chronic phase

Tubulointerstitial lesions

Control: Day 5

AA rats: Day 5

Control: Day 35

AA rats: Day 35
Semiquantitative score of tubulointerstitial injury

Phases: Acute          Chronic

* p<0.05, ** p<0.01, *** p<0.005  AA vs control group
PTEC proliferation ↔ Ki-67 immunostaining

Control: Day 3

AA rat: Day 3

AA rat: Day 35

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Controls</th>
<th>AA rats</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>2</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>3</td>
<td>***</td>
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<td>5</td>
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<td>7</td>
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<td>***</td>
</tr>
<tr>
<td>10</td>
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<td>14</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>18</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>35</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Phases: Acute vs Chronic

*p<0.05, **p<0.01, ***p<0.005 AA vs control group
Dedifferentiation of PTEC

Control: Day 3

AA rat: Day 3

AA rat: Day 10

AA rat: Day 10

AA rat: Day 35

Vimentin / Ki-67
Key events of epithelial to mesenchymal transition (EMT)

1. Loss of epithelial adhesion
2. De novo \(\alpha\)SMA expression and actin reorganization
3. AngII blockade
4. Enhanced cell migration and invasion
5. TBM disruption
6. ROCK inhibition
7. MMP-9 reduction
8. tPA-/-

TGF-\(\beta\) alone is capable of inducing epithelial cells to undergo all four steps

Zvaifler . Arthritis Research & Therapy 2006
Tubular basement membrane integrity

Control: Day 2

AA rat: Day 2

AA rat: Day 5

Basement membrane denudation score

Time (days)

*p<0.05, **p<0.01, ***p<0.005 AA vs control group
Myofibroblast phenotype: $\alpha$-SMA immunostaining

Control: Day 35

AA rat: Day 35

Myofibroblast phenotype: α-SMA immunostaining

Control: Day 35

AA rat: Day 35

Myofibroblast phenotype: $\alpha$-SMA immunostaining

Control: Day 35

AA rat: Day 35

Activation of resident fibroblasts

Paritubular fibrosis

Renal tissue expression of TGF-β

Control: Day 3

AA rat: Day 3

AA rat: Day 10

AA rat: Day 35
### Urinary excretion rate of proinflammatory and profibrosing cytokines

<table>
<thead>
<tr>
<th>Variables</th>
<th>Day 10</th>
<th>Day 35</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>AA rats</td>
</tr>
<tr>
<td>(ng/mmol Cr)</td>
<td></td>
<td></td>
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<tr>
<td>IL-1α</td>
<td>8.35</td>
<td>8.31</td>
</tr>
<tr>
<td></td>
<td>(4.65-9.26)</td>
<td>(5.76-17.0)</td>
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<tr>
<td>TNF-α</td>
<td>0.19</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>(0.00-0.38)</td>
<td>(0.00-12.4)</td>
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<tr>
<td>IFN-γ</td>
<td>0.08</td>
<td>0.23</td>
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<td>(0.08-1.01)</td>
<td>(0.00-2.19)</td>
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<td>MCP-1</td>
<td>119</td>
<td>74.7</td>
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<tr>
<td></td>
<td>(84.2-145)</td>
<td>(36.7-110)</td>
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<td>IL-4</td>
<td>3.56</td>
<td>9.3</td>
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<td></td>
<td>(0.00-6.00)</td>
<td>(0.00-16.2)</td>
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<tr>
<td>Active TGF-β</td>
<td>0.81</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td>(0.00-1.91)</td>
<td>(1.52-8.41)</td>
</tr>
</tbody>
</table>

*P* < 0.05, **P** < 0.005.

Pozdzik et al. *Nephrol Dial Transplant* 2008
Distribution of the monocyte/macrophage infiltrate
Semiquantitative score
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score
Distribution of the monocyte/macrophage infiltrate
Semiquantitative score
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score

Cortex
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score

Phases: Aiguë                          Chronique

Cortex

Outer stripe of outer medulla (OSOM)
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score

Cortex

Outer stripe of outer medulla (OSOM)

Inner stripe of outer medulla (ISOM)
Distribution of the monocyte/macrophage infiltrate

Semiquantitative score

Phases: Aiguë                          Chronique

Cortex

ED-1 immunomarquage (amas/ champ)

Outer stripe of outer medulla (OSOM)

Inner stripe of outer medulla (ISOM)
Distribution of the monocyte/macrophage infiltrate
Semiquantitative score

**Phases:**
- *Aiguë*
- *Chronique*

**Cortex**
- **Outer stripe of outer medulla (OSOM)**
- **Inner stripe of outer medulla (ISOM)**

*ED-1 immunomarquage (amas/ champ)*

- *p<0.05, **p<0.01, AA vs controls. N = 6 rats/group*
Distribution of the monocyte/macrophage infiltrate
Semiquantitative score

**Cortex**

**Outer stripe of outer medulla (OSOM)**

**Inner stripe of outer medulla (ISOM)**

*p<0.05, **p<0.01, AA vs controls. N = 6 rats/group*
Distribution of the monocyte/macrophage infiltrate
Semiquantitative score

**Phases:**
- Aiguë
- Chronique

**Distribution of the monocyte/macrophage infiltrate**

**Cortex**

**Outer stripe of outer medulla (OSOM)**

**Inner stripe of outer medulla (ISOM)**

*ED-1 immunomarquage (amas/ champ)*

*Temps (jours)*

*0 5 10 15 20 25*

*N = 6 rats/group*

*p<0.05, **p<0.01, AA vs controls.*
Interstitial T lymphocytes

**Anti-CD3**
- Control
- AA

**Anti-CD20**
- Control
- AA

**Anti-CD45RC**
- Control
- AA

**Anti-CD8**
- Control
- AA
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4. Perspectives of research
Translational nephrology is ongoing!
Translational nephrology is ongoing!

- Physiopathological mechanisms of renal fibrosis, including the potential role of immunocompetent cells
Translational nephrology is ongoing!

- Physiopathological mechanisms of renal fibrosis, including the potential role of immunocompetent cells

- Therapeutic strategies to reduce the onset and/or progression of fibrosis
Translational nephrology is ongoing!

- Physiopathological mechanisms of renal fibrosis, including the potential role of immunocompetent cells

- Therapeutic strategies to reduce the onset and/or progression of fibrosis
Translational nephrology is ongoing!

- Physiopathological mechanisms of renal fibrosis, including the potential role of immunocompetent cells

- **Therapeutic strategies** to reduce the onset and/or progression of fibrosis

  From bench to bedside…
Thank you to…

- Nephrology, Pathology and Urology depts, Erasme university hospital, ULB
- Experimental nephrology unit, ULB
- Institute of Pharmacy, ULB
- Inserm U785, Paul Brousse
- German Cancer Institute, Heidelberg, Germany
- Molecular Toxicology Unit, Sutton, UK
- Nephrology dept, AZ-VUB