Working together to stop the ongoing tragedy of rabies!

Make rabies history!
Rabies: a neurotropic virus

LONG INCUBATION PERIOD: 2 months (2 w / 7 y)

- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS

- transsynaptic pass
- transcrip/replication
- retrograde transport (25 mm / day)
- entry

- replication

- microtubule based motors

- candidates receptors
  - nAchR
  - p75NTR
  - NCAM
Rabies: a neurotropic virus

SHORT SYMPTOMATIC PERIOD (1 week)
• neuronal dysfonctions (neurotransmitters, ...)
• to non-neuronal tissues (salivary glands)
• transmission (bite, aerosol)

transsynaptic pass

transcript/replication

retrograde transport (25 mm/day)

entry

dendrites

soma

neuron

replication

microtubule based motors

candidates receptors

nAchR  p75NTR  NCAM
Tools for prevention/therapy

Pasteur’s vaccine
rabid rabbit spinal cord -> dessiccated

in 2015

*human vaccines* (prevention + therapy)

Not recommended by WHO

- cell culture: safe + efficient (expensive?)

*animal vaccines* (prevention)

- nervous tissue (injection)
- cell culture (injection)
- attenuated/recombinant (oral, wildlife)

No efficient antiviral
## Rabies and antivirals, an empirical arsenal

<table>
<thead>
<tr>
<th>Drug/combination</th>
<th>In vitro</th>
<th>Animal</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-interferon:</td>
<td></td>
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<tr>
<td>Ribavirin (purine analogs, AraC):</td>
<td></td>
<td>mouse</td>
<td>fox</td>
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<tr>
<td>Interferon &amp; vidarabine:</td>
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<tr>
<td>Dolman &amp; Charlton 1987 <em>Can J Neurol Sci</em> 14</td>
<td></td>
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<tr>
<td>Ketamine (antagonist NMDA receptor):</td>
<td></td>
<td>rat</td>
<td>stereotax</td>
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<tr>
<td>1991 <em>Antiviral Chem Chemother</em> 2:9-15</td>
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<td>Heteropolyanions:</td>
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<tr>
<td>Pepin &amp; Blancou 1985 <em>Archiv. Virol</em> 83</td>
<td></td>
<td>fox</td>
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<tr>
<td>Corticosteroids:</td>
<td></td>
<td></td>
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<tr>
<td>Enright et al 1970 <em>Can J Microbiol</em> 16</td>
<td></td>
<td>mouse</td>
<td></td>
</tr>
<tr>
<td>Amantadine, rifampicin, cinnabarin, chloroquin, neurotrophin, cholchicin, vinblastin, asobic acid:</td>
<td></td>
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<tr>
<td>Natural / semi-synthetic polymers, phenolic compounds plant extracts (<em>red beans from South-America</em>):</td>
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<tr>
<td>Antisens oligonucleotides, siRNA:</td>
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</tbody>
</table>
Survival after treatment of rabies with induction of coma


Scholar-athlete aged 15
Future veterinarian
Picked up downed bat
Laceration L index finger
No post-exposure prophylaxis
One month incubation

Amantadin
Ribavirin

Ketamine
Benzodiazepines
Ventilator
Hospitalization
Incubation
Temperature > 38.9°C
SAH or diabetes insipidus
Sit > 10 min
Stand with assistance
Use walker with assistance
Feed self
Write name
Use sign language
Speak with >75% intelligibility

Hospital Day
-36  1  9  32  76  131
Rabies and antivirals

Advantages

• No existing therapy (vaccine and RIG are « preventive »)
• long incubation period (2 weeks to 7 years: 2 months)
• increased interest (Millwauk ee protocol)

Disadvantages

• tropism for the neurone (difficult to reach)
• neglected disease (poor countries)

Development of large spectrum antivirals targeting common targets to negative strand RNA viruses
Two main strategies to find anti-rabies molecules

**Combinatory approach**

High-throuput screening assays on libraries of molecules

- **compounds**
- **siRNA**
- **peptides**…

**Candidates molecules**

**Validation by in vitro tests**

- Inhibition of rabies infection
- Inhibition of entry assay
- Inhibition of a minireplicon (replication)

**Cognitive approach**

Design of molecules based on known functional interactions

- Lentivirus vector encoding GFP/Luc
- Rabies G-protein

**Rabies virus**
Inhibition of entry

LONG INCUBATION PERIOD: 2 months (2 w / 7 y)
- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS

candidates receptors
nAchR, p75NTR, NCAM

transsynaptic pass
transcrip/replication
retrograde transport (25 mm / day)
entry

microtubule based motors

repetation

Dynein
Base
Head
minus (-) end
DLC8
Light and intermediate chains

nAchR
p75NTR
NCAM
Dermaseptins

- cationic peptides
- 30 aa long, rich in lysine
- form amphiphilic $\alpha$ helix
- destabilize / disrupt membrane

Antimicrobial potential

- **Bacteria**  (Navon-Venezia & al, 2002)
- **yeast**  (Coote & al, 1998)
- **protozoan**  (Brand & al, 2002)

**Enveloped Viruses**

- **HSV**  (Belaid & al 2002)
- **HIV**  (Lorin & al 2005)
Anti-rabies activity of dermaseptins

**in vitro** (co-infection)

- **AntiRABV activity mostly in pos. 1-5**
- **Lysine in position 4 is important**

**in vivo**

(1h post-infection same site)

- 10 mice/group

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% mouse survival</th>
<th>% infection inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S4 100 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4 200 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4M4K 100 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4M4K 200 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4(1-16) 100 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4(1-16) 200 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Virus only</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>S4 100 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>S4 200 µg</td>
<td>100%</td>
<td>0%</td>
</tr>
</tbody>
</table>

- **S4 / S4M4K > S3 / S41-16 >> S46-26**
- **S4M4K > S41-16**

- **S4 not efficient post-exposure (size ? stability ? penetration ?)**
LONG INCUBATION PERIOD: 2 months (2 w / 7 y)

- peripheral inoculation (muscle, ...)
- nerve endings (neuro-muscular junction)
- from neurone to neurone up to CNS

Inhibition of replication

candidates receptors
nAchR  p75NTR  NCAM

replication

microtubule based motors

transcription/replication

retrograde transport (25 mm / day)

entry

transynaptic pass

soma  neuron  axone  dendrites
A similar transcription/replication complex (RNP)

**Virion**

- Rabies v.
- Measles v.
- Ebola v.

**RNP**

- Rabies RNP

**Template**

- RNA genome (-)
- + nucleoprotein N

**Enzymes**

- phosphoprotein P (cofactor)
- RNA polymerase L

Exemple 1: Peptides targeting the RNP complex

Combinatory approach

Screening of random peptide libraries for their affinity to the RNP

Candidate peptides

- 2-hybrid (Real et al. 2004)

target: P protein
2 lyssavirus

rabies  Mokola

Functional tests:
- Inhibition of a minireplicon (RNP)
- Inhibition of viral infection
combinatory library of auto-contrast peptides in *S. cerevisiae* (2-hybrid)

Toxins: conotoxins (mollusques); defensins (mammals) 
*constraint through disulfures bridges (cysteines)*

Cys library (26 a.a) $1 \times 10^7$ independent peptides

C-2x-C-5x-C-6x-C-5x-C-2x-C- 

Gal4 AD

Antimicrobial properties: lebocines, apidaecines (insects) 
*conformational constraint through prolines (turns)*

Pro library (29 a.a) $3 \times 10^7$ independent peptides

PP-5x-P-5x-PPP-5x-P-5x-PP - 

Gal4 AD
Inhibition of RNP transcription (minireplicon) 11
Protein chip analysis (inhibition N/P interaction) 8
Inhibition of cell infection 4

Selection steps

Peptide libraries

2-hybrid screening

P-Rab partners

P-Mok partners

46.5% identity

In silico analysis (phylogeny, groups)

Inhibition of RNP transcription (minireplicon)

Protein chip analysis (inhibition N/P interaction)

Inhibition of cell infection

Candidates

8.55 \times 10^7

755
# inhibition of Neuro-2A cell infection

<table>
<thead>
<tr>
<th>Peptide</th>
<th>% inhibition infection</th>
<th>% inhibition replication (luciferase activity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2</td>
<td>89 %</td>
<td>97.9 %</td>
</tr>
<tr>
<td>C6</td>
<td>83 %</td>
<td>97.2 %</td>
</tr>
<tr>
<td>C8</td>
<td>78 %</td>
<td>98.9 %</td>
</tr>
<tr>
<td>P16</td>
<td>71 %</td>
<td>96.3 %</td>
</tr>
</tbody>
</table>

(Real et al., J. Virol 2004, 78: 7410-7)
Exemple 3: Peptides targeting the RNP complex

**Combinatory approach**

- Functional tests:
  - Inhibition of a minireplicon (RNP)
  - Inhibition of viral infection
- Screening of random peptide libraries for their affinity to the RNP
- 2-hybrid (Real et al. 2004)
- Phage display (unpublished)

**Cognitive approach**

- Design of peptides based on known interactions in the RNP
- Candidate peptides

Target: P protein 2 lyssavirus
- Rabies
- Lagos bat
The rabies virus ribonucleocapsid as a target


Pos. 70-100

Pos. 4-42

Phosphoprotein

P42 → P60

P - P dimers
P - N°
P - L

N-ARN

N-N

RNA

N terminal sub-domains
C terminal sub-domains

L

NH2
COOH

NH2
COOH

NH2

POS. 4

4

P-N°

40

70

P-L

100

297
• P60 maintains its inhibitory effect up to 55h post-transfection
• the inhibitory effect of P42 progressively decreases after 24h

\[ \rightarrow \text{not due to peptide degradation} \]

Inhibition of rabies virus infection by synthetic Tat-peptides

Control

P42-Tat

P60-Tat

→ P42-Tat (≈60%) < P60-Tat (≈80%)
→ dose dependent effect

T=0 : Infection
T=1h : 10μM peptide → T=14h

Tat-peptides inhibit rabies virus infection in human neurons

SK-N-SH neuroblastoma

SK-N-SH CP

Infected cells

P42

P1-20 + 20-40 + 40-60

Alexa Fluor 488

peptide

Tat

peptide

Tat

Crystal structure of a decameric form of the NΔ210-P60 complex

P binding hinders RNA binding and self-assembly of soluble N°

Conclusions
There is a need to develop rabies antivirals / therapies

1. Post-exposure vaccination
   → Shorten schedule (one week)
   → Reduce vaccine volume required (ID rather than IM)

1. Replacing HRIG and ERIG (shortage, cost)
   → Cocktail of human(ized) Mabs
   → Dermaseptins

2. Developing large spectrum (vaccines) antiviral strategies
   → Random screening (molecules, peptides)
   → Cognitive approach
     ✓ Further dissection of interacting domains
     ✓ Drug design : peptido-mimicry
Ebola Yambuku: 1976

Pr Pierre SUREAU, Institut Pasteur
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