Doxycycline trials in CJD and FFI

Gianluigi Forloni
Neuroscience Department
Istituto di Ricerche Farmacologiche “Mario Negri”
Tetracyclines affect prion infectivity

Gianluigi Forloni*, Selina Iussich†, Tazeen Awan†, Laura Colombo*, Nadia Angeretti*, Laura Girola*, Ilaria Bertani*, Giorgio Poli†, Maria Caramelli†, Maria Grazia Bruzzone†, Laura Farina†, Lucia Limido†, Giacoma Rossi†, Giorgio Giaccone†, James W. Ironside§, Orso Bugiani†, Mario Salmoña*, and Fabrizio Tagliavini†

263K scrapie strain

No treatment

Tetracycline

A

Number of animals

10

9

8

7

6

5

4

3

2

1

0

70

130

190

250

Days after inoculation

B

Number of animals

8

7

6

5

4

3

2

1

0

100

150

200

250

300

350

400

Days after inoculation

A

B

C

No treatment

Tetracycline

No treatment

Tetracycline
Tetracycline reduces the PK resistance of PrP\textsuperscript{Sc} from sCJD

Tagliavini et al — JMB 2000
Summary

TETRACYCLINES

1. bind to PrP peptides
2. prevent aggregation of PrP peptides
3. disrupt amyloid fibrils generated by PrP peptides
4. abolishes neurotoxicity of PrP peptides
5. revert protease resistance of PrP peptides and PrPSc from sCJD, vCJD, BSE and 263K scrapie
Conclusions from *in vitro* and *in vivo* studies

The anti-prion activity of tetracyclines seems to be related to a direct interaction with the disease-associated forms of PrP, resulting in disruption of PrP aggregates and reversion of protease resistance.
# Anti-fibrillogenic activity of tetracyclines

<table>
<thead>
<tr>
<th>Peptides/proteins</th>
<th>Inhibition of aggregation rate</th>
<th>Disruption of fibrils</th>
<th>Toxicity in-vitro</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrP 106-126, PrP 82-146, PrP&lt;sup&gt;sc&lt;/sup&gt;</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>[14, 27]</td>
</tr>
<tr>
<td>Abeta (1-42)</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>[16]</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>[35,36]</td>
</tr>
<tr>
<td>W7FW14F Apomyoglobin</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>[34]</td>
</tr>
<tr>
<td>Amylin</td>
<td>Yes</td>
<td>n.d.</td>
<td>n.d.</td>
<td>[55]</td>
</tr>
<tr>
<td>Huntingtin</td>
<td>Yes</td>
<td>n.d.</td>
<td>n.d.</td>
<td>[40]</td>
</tr>
<tr>
<td>α&lt;sub&gt;2&lt;/sub&gt; macroglobulin</td>
<td>Yes</td>
<td>Yes</td>
<td>n.d.</td>
<td>[*]</td>
</tr>
<tr>
<td>α-synuclein</td>
<td>Yes</td>
<td>Yes</td>
<td>n.d.</td>
<td>[56]</td>
</tr>
<tr>
<td>Poli (A) binding Protein nuclear 1</td>
<td>Yes</td>
<td>n.d</td>
<td>Yes</td>
<td>[37-39]</td>
</tr>
</tbody>
</table>

Forlioni et al 2009
Some tetracycline derivatives (e.g. 4-epi-tetracycline) have low antimicrobial activity, but are very effective on PrP peptides.

Possible dissociation between antibiotic and anti-prion capability.
doxycycline in CJD

Observational study in patients from “Carlo Besta” Neurological Institute - Milano

Diagnostic Protocol

- PRNP analysis
- CSF analysis including 14-3-3 and tau
- EEG – polygraphic recording
- Electroretinogram
- Evoked Potentials
- MRI

“probable” or “definite” CJD
CJD patients

- **Doxycycline**  
  (oral dose of 100 mg/day)  
  \( n = 21 \)

- **No treatment**  
  \( n = 77 \)
## Characteristics of CJD patients treated or untreated with doxycycline

<table>
<thead>
<tr>
<th>Doxy</th>
<th>N</th>
<th>Sex</th>
<th>PRNP 129</th>
<th>Age at Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
<td>MM</td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>8</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(38% - 62%)</td>
<td></td>
<td>(76% - 19% - 5%)</td>
</tr>
<tr>
<td>No</td>
<td>77</td>
<td>44</td>
<td>33</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(56% - 44%)</td>
<td></td>
<td>(53% - 22% - 25%)</td>
</tr>
</tbody>
</table>

*Pearson Chi-Square* \( p=0.14 \)  \( p=0.09 \)

*t-test for equality of means* \( p=0.02 \)
Survival in doxycycline-treated and untreated sCJD and fCJD

Cumulative survival

Disease duration (months)

Doxy

Untreated

Survival time (mo)

mean ± se median ± se

19.7 ± 3.8 13.0 ± 4.2

8.5 ± 0.8 6.0 ± 0.7

Log Rank test  p< 0.001
Multivariate survival analysis in sCJD and fCJD patients

Cox proportional hazards model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted Relative Risk (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male M</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Female F</td>
<td>1.02 (0.64-1.62)</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>PRNP codon 129</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM MM</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>MV MM</td>
<td>0.68 (0.38-1.21)</td>
<td>0.19</td>
</tr>
<tr>
<td>VV MM</td>
<td>0.60 (0.32-1.14)</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Age at onset</strong></td>
<td>1.03 (1.00-1.05)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Doxycycline</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.41 (0.21-0.79)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* continuous
Survival of doxycycline-treated and untreated CJD patients matched for gender, codon 129, PRNP mutations and age at onset

Survival time (mo)  

<table>
<thead>
<tr>
<th></th>
<th>mean ± se</th>
<th>median ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxy</td>
<td>20.2 ± 3.8</td>
<td>13.9 ± 3.8</td>
</tr>
<tr>
<td>Untreated</td>
<td>9.6 ± 2.1</td>
<td>6.1 ± 0.5</td>
</tr>
</tbody>
</table>

Log Rank test  \( p=0.01 \)
Survival in doxycycline-treated and untreated CJD patients verified at autopsy \((n=32)\)

**Survival time (mo)**

<table>
<thead>
<tr>
<th></th>
<th>mean ± se</th>
<th>median ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxy</td>
<td>11.4 ± 3.0</td>
<td>9.0 ± 3.5</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.6 ± 0.9</td>
<td>4.0 ± 0.5</td>
</tr>
</tbody>
</table>

Log Rank test \(p=0.04\)

**Table: MM1 MM2 MV1 MV2 VV2**

<table>
<thead>
<tr>
<th></th>
<th>MM1</th>
<th>MM2</th>
<th>MV1</th>
<th>MV2</th>
<th>VV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxy</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Untreated</td>
<td>14</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>
Survival: treated vs. non-treated Patients in German study

- Doxycycline treatment >30 days
- Median DOXY: 292 d (n=21)
- Median CRTL: 169 d (n=581)
- The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference ($p = <0.001$)

Data from I. Zerr et al
Summary: Survival

![Bar chart showing survival rates for different categories]

- Total
- MM
- MV
- VV

Legend:
- DOXY
- CRTL
PK resistance of PrP from different CJD subtypes

The samples have been digested for 7 hours at 37°C
PK resistant of PrP from different CJD subtypes

VV2

MV2

MV1

MM1
A randomized, double-blind pilot study for the evaluation of efficacy and tolerability of doxycycline administered by oral route vs. placebo in patients affected by Creutzfeldt-Jakob disease

Protocol Code: CJD200501  EudraCT No.: 2006-001858-27

Coordinating Centre: Fondazione IRCCS - Istituto Neurologico “Carlo Besta”, Milano, I

Principal Investigator: Fabrizio Tagliavini, MD
Head of the Department of Neurodegenerative Diseases
Director of the Division of Neurology V – Neuropathology

Protocol Authors

• Fabrizio Tagliavini

• Alfredo Martini
  Head of Clinical Research and Development Service, Istituto Neurologico Carlo Besta

Study (protocol and ICF) approved by Coordinating Centre EC/IRB on 16th May 2006
Summary of study design

- Double-blind
- Randomized versus placebo (Mario Negri Institute)
  gender - age - time from onset - *PRNP* codon 129
- 2 parallel groups
- Study population: 60 patients affected by sporadic or familial CJD, classified as definite or probable (WHO criteria)
- Multicentre
Clinical centers involved

- Milano  Istituto Neurologico Carlo Besta  F. Tagliavini  G. Marcon  A. Martini
- Torino  Ospedale Maria Vittoria, Neurologia  C. Buffa  D. Imperiale
- Genova  Clinica Neurologica II, Università  G.L. Mancardi
- Parma  Clinica Neurologica Università  V. Pietrini
- Napoli  Clinica Neurologica, 2ª Università  G. Puoti

Centers for analysis and support

- Milano  Istituto di Ricerche Farmacologiche Mario Negri  G. Forloni U. Lucca M. Tettamanti M. Salmona
- Roma  Istituto Superiore di Sanità  M. Pocchiarri
Inclusion criteria

• male or female out-patients
• aged 18 to 80 years inclusive
• diagnosis of “probable” or “definite” CJD (WHO)
• first symptoms by no more than 6 months
• a brain MRI within 6 months and an EEG within 3 months
• no conditions known to be contraindications to the use of tetracyclines
• written informed consent

Exclusion criteria

• end-stage liver, heart or renal disease
• active malignancy
• female subjects who are pregnant or lactating
• subjects who have received an experimental drug or have participated in a clinical trial within 3 months prior to screening
• employees of the investigator or study centre
Treatments

• **Doxycycline** at a oral daily dose of 100 mg (30 patients) or with identical **Placebo** by oral route at the same dosing schedule (30 patients), under double-blind conditions.

• **Treatment** with doxycycline or placebo will start at patient entry into the study (randomization day) and will continue throughout the study, until patient *exitus* or until it will be possible on the bases of patient clinical status.

• Every concomitant and/or supportive therapy will be admitted.
Efficacy evaluation

• **Primary efficacy variable**: “survival time” from the time of randomization

• **Secondary end-points**
  • “time to loss” of autonomous feeding (if appropriate)
  • “time to loss” of sphincters control (if appropriate)
  • “time to reaching the clinical stage of akinetic mutism” (if appropriate)
  • number of patients showing a survival time of more than 12 months from the onset of CJD (number of responders)
Efficacy evaluation (continued)

Additional endpoints

- **Cognitive status**: MMSE/SMMSE (if appropriate)
- **Neurological status**: Comprehensive index for neurological deterioration (NDI)
- **Level of Consciousness**
- **Disability** scale (Barthel index)
- **Behavioural** rating scale (NPI)
- Volumetric brain **MRI** (selected patients)
- **CSF**: concentration of tau protein (selected patients)
- **24-hour EEG** recording (selected patients)
- **3-day Actigraphy** recording (selected patients)
Time and events schedule

Screening Visit (continued)

• 24-h polygraphic EEG (if possible)
• 3-day Actigraphy (if possible)
• CSF: routine tests; 14-3-3 and tau concentration (Besta)
• pre-study medication (CJD specific therapy excluded)
• pre-study CJD supportive therapy
• Adverse Events
• informed consent
• inclusion/exclusion criteria
• randomization (Negri): age - sex - time from onset - PRNP codon 129
• study medication dispensing
Time and events schedule

First Control
at Hospital (if possible) after 2 months of treatment
Same clinical and laboratory analyses as for the screening visit

Subsequent controls
Second and third control at home (or nursing home) after 4 and 6 months of treatment; subsequent controls at 3-month intervals up to death. These controls are carried out by a Neurologist from Carlo Besta Institute. Same clinical evaluation as for the screening visit.
Update April 1st, 2011

- Randomized patients 59 (36 F, 23 M)
- Treated patients 56 (34 F, 22 M)

Treated patients > 30 days 38 (28 F, 10 M)

- Age range 41-79 (mean 63.8 ± 1.1)
  - 30MM (51%)
  - 19MV (32%)
  - 10VV (17%)

- PRNP Mutation
  - 5 E200K
  - 7 V210I

- Mean Survival: 9.24 ± 0.98 months (59)
- Adverse events: none
38 patients treated ≥ 1 month

• **Age**  range 41-79 (mean 64. ± 1.41 )

• **PRNP Codon 129**
  - 20 MM (52.6%)
  - 13MV (34.2%)
  - 5 VV (13.2%)

• **PRNP Mutation**
  - 3  E200K
  - 4  V210I

**Mean Survival:** 11.2 ± 1 months (onset)
**Mean Survival:** 8.05 ± 0.94 months (treat)

**Adverse events:** none
Milano

• Randomized patients 37 (21F, 16 M)
• Treated patients 34 (19 F, 15 M)
• Treated patients > 30 days 28 (16F, 12 M)

• Age range 41-77 (mean 61.7 ± 2.2)

• PRNP Codon 129 20 MM (58%)
  10 MV (30%)
  4 VV (12.0%)

• PRNP Mutation 2 E200K
  3 V210I

Mean Survival: 10.23 ± 1.5 months (onset)
Mean Survival: 7.7 ± 1.5 months (treat)
• Randomized patients 17

• Treated patients 15 (11 F, 4 M)

• Treated patients > 30 days 9 (7 F, 2 M)

• Age range 41-76 (mean 66.9 ± 2.5)

• PRNP Codon 129
  - 6 MM (30.3%)
  - 8 MV (53.3%)
  - 3 VV (15.3%)

• PRNP Mutation
  - 3 E200K
  - 1 V210I

Mean Survival: 7.2 ± 2.0 months (onset)
Mean Survival: 6.8 ± 2.5 months (treat
Survival of Treatment: Survival proportions

Number of rows 38
# of blank lines 0
# rows with impossible data 0
# censored subjects 0
# deaths/events 38
Median survival 6
Survival of Onset: Survival proportions

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rows</td>
<td>38</td>
</tr>
<tr>
<td># of blank lines</td>
<td>0</td>
</tr>
<tr>
<td># rows with impossible data</td>
<td>0</td>
</tr>
<tr>
<td># censored subjects</td>
<td>0</td>
</tr>
<tr>
<td># deaths/events</td>
<td>38</td>
</tr>
<tr>
<td>Median survival</td>
<td>10</td>
</tr>
</tbody>
</table>
Survival of Treatment F vs M: Survival proportions

Comparison of Survival Curves

Log-rank (Mantel-Cox) Test
Chi square 15.83
df 1
P value < 0.0001
P value summary ***
Are the survival curves sig different? Yes

Gehan-Breslow-Wilcoxon Test
Chi square 16.26
df 1
P value < 0.0001
P value summary ***
Are the survival curves sig different? Yes

Median survival
F 8.000
M 2.000
Ratio 4.000
95% CI of ratio 3.514 to 4.486

Hazard Ratio
Ratio 0.08635
95% CI of ratio 0.02584 to 0.2886
Survival of Onset F vs M: Survival proportions

Comparison of Survival Curves

Log-rank (Mantel-Cox) Test
Chi square 15.44
df 1
P value < 0.0001
P value summary ***
Are the survival curves sig different? Yes

Gehan-Breslow-Wilcoxon Test
Chi square 15.49
df 1
P value < 0.0001
P value summary ***
Are the survival curves sig different? Yes

Median survival
F 10.50
M 6.000

Ratio 1.750
95% CI of ratio 1.264 to 2.236

Hazard Ratio
Ratio 0.09151
95% CI of ratio 0.02776 to 0.3017

Percent survival

Number of rows | F | M
---|---|---
# of blank lines | 38 | 38
# rows with impossible data | 10 | 28
# censored subjects | 0 | 0
# deaths/events | 28 | 10
Median survival | 10.5 | 6
Female vs Male

<table>
<thead>
<tr>
<th>Mean survival</th>
<th>Treatment</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (28)</td>
<td>11.0 ± 1.4</td>
<td>14.5 ± 1.45</td>
</tr>
<tr>
<td>Males (10)</td>
<td>3.4 ± 1.0</td>
<td>6.5 ± 0.95</td>
</tr>
</tbody>
</table>
CJD patients: survival from treatment

Percent survival vs. Time (days)
Conclusions

A randomized double blind clinical trial vs placebo in CJD is feasible

The number of patients (59) recruited within 3.5 years is acceptable although inferior at the prediction (60 in 2 yrs)

Only 41 patients (69%) received the treatment for at least 1 month to allow the evaluation of efficacy

Doxycycline is well tolerated, none adverse events were recorded during the treatment

Two similar studies are started in France and Germany
“Efficacité et tolérance de la Doxycycline dans le traitement des maladies humaines à prions : un essai randomisé en double aveugle contre placebo”.
Short name « Doxyprions ».

Recruited 47 patients (36 sporadic CJD).

Started in January 2009 and they intend to include until the end of 2011

Coordinator: Jean-Philippe Brandel, Paris Groupe Hospitalier Pitie-Salpetriere
A randomized, double-blind study for the evaluation of the efficacy of orally administered doxycycline versus placebo in patients affected by sporadic Creutzfeldt-Jakob disease

funding by BMBF- German Ministry for education and research

start: 1.10.2009, estimated duration 42 months

recruited: 12 for double blind study and 40 for observational study

Coordination by Dr. Inga Zerr, Gottingen
Fatal familial insomnia: preventive treatment with doxycycline in risk individuals

Telethon application # GGP 10208

Coordinator: Gianluigi Forloni (Istituto Mario Negri, Milano)

Partner 1: Ignazio Roiter (USSL 9 Treviso)

Partner 2: Fabrizio Tagliavini (Istituto Carlo Besta, Milano)

Treatment: Doxycycline Iclate (Bassado) 100 mg/ Kg /day

Time of observation: 120 months

Neurological analysis every two years

Primary outcome: survival
Prevention treatment

Symptoms
- none
- severe

PrP accumulation

TIME

Presymptomatic phase
Disease Onset

Clinical phase
Clinical Onset

PrP accumulation

Prevention

Treatment
Genealogy of FFI family from Treviso area

“ASSOCIAZIONE FAMILIARI INSONNIA FAMILIARE FATALE - MALATTIE DA PRIONI”. [www.afiff.org](http://www.afiff.org)/ Dr. Ignazio Roiter

Fatal Familial Insomnia Families Association - Prion Diseases
Eperimental design

• All individuals aged between 40 and 52 yrs have accepted to participate to the study.

• This population comprises 11 carriers of the FFI mutation and 19 non-carriers.

• The individuals with mutation will be chronically treated with doxycycline at an oral daily dose of 100 mg, while the individuals without mutation will be treated with placebo.

• The treatments will be carried out under double-blind condition.
Study population

large FFI kindred from Treviso area

• Genetic analysis of 89 non-symptomatic family members older than 18 years unveiled that 22 subjects carry the D178N-129M PRNP mutation.

• Only individuals born between 1958 and 1970 (age range 40-52 years) will be enrolled in the study, as the onset of disease in this family peaks at 53 years of age.
Experimental design

• Basal Evaluation and follow-up every two years will include:
  Physical and neurological examination
  Neuropsychological assessment
  Polysomnographic EEG recording/Actigraphy
  3T MRI and FDG-PET
  Autonomic Nervous System Assessment

• Primary efficacy variable
  > 50% of individuals carrying the mutation without clinical disease after 10 years
Development of the study

Protocol submission to EC  March 2011

Start recruitment                      June 2011

Start treatment                         July 2011

1° Follow up                             July 2013

2°-5° Follow up                        2015- 2021

End of the observation           June 2022