Bilirubin and Brain: New Aspects in Toxicity, Therapy, Models and Protection

TOXICITY

- ✓ Epigenetic of hyperbilirubinemia
- ✓ Blood brain interfaces in hyperbilirubinemia

(N. Strazielle, J-F. Ghersi-Egea, FLUID, Inserm U1028 - CNRS UMR5292, Lyon, France)

THERAPY

- *In vivo* evaluation of a pharmacological approach to bilirubin induced neurotoxicity
- Phototherapy, is really so safe?

MODELS & PROTECTION

- ➢ Parkinson disease OBCs model
- ➢ Protective UCB threshold in Neurodegeneartive Diseases (NDs)
- ➢ Severe phenotype in Gunn rat

S. Gazzin, C. Tiribelli, A. Bramante, A. Lorenzon – CBM Scarl

The Epigenetic of Hyperbilirubinemia

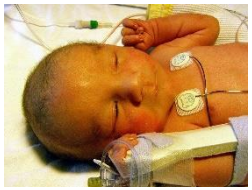
(toxicity)

Is bilirubin able to exert an epigenetic control?

Is it part of the toxic neurological action of the pigment?

The experimental scheme:

1. Hyperbilirubinemic jj *vs.* normobilirubinemic Nj age-matched Gunn rat
2. Quantification of Histone 3 Lysine 14 acetylation (H3K14Ac) level by Western blot
3. Chromatin Immunoprecipitation and Gene sequencing (ChIP-Seq)
4. Gene ontology analysis
5. Brain histology analysis
6. Real Time PCR (RTqPCR)



E. Vianello, S. Gazzin
C. Tiribelli

A. Bramante, A. Lorenzon
CBM Scarl, TS

A. Dardis, S. Zampieri
Dip. Malattie Rare
S. Maria della Misericordia, UD

P. Ostano
Fondazione Edo ed Elvo Tempia Valenta
Università degli Studi di Torino

C. Bottin, F. Zanconati, T. Marcuzzo
Dip. Anatomia Patologica
Ospedale di Cattinara, TS

The Epigenetic of Hyperbilirubinemia

(toxicity)

Age dependent modulation of the H3K14Ac levels in 3 region of the developing Gunn rat's brain

ChIP-Seq:
1886 genes differentially expressed by hyperbilirubinemia (through H3K14aC)

Gene Ontology:
45% of genes are involved in "brain development" (differentiation, neurites/dendrites out-growth, migration, etc.), confirmed by RTqPCR

Histology:
Relevant evidences of an aberrant brain' s development

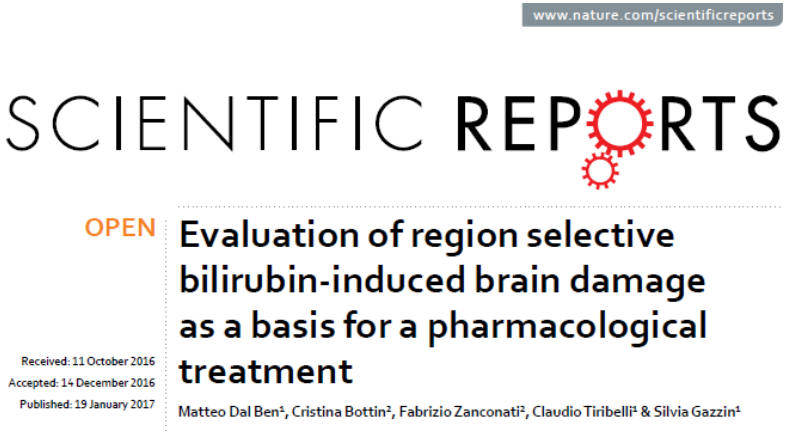
Relevant epigenetic alteration in hyperbilirubinemia, with a strong developmental genetic impact

In vivo evaluation of a pharmacological approach to bilirubin induced neurotoxicity

(from toxicity to therapy)

M. Dal Ben
M. Montrone
S. Gazzin
C. Tiribelli

Background:



C. Bottin
F. Zanconati
Dip. Anatomia Patologica
Ospedale di Cattinara

1) Bilirubin induced damage is triggered by the simultaneous activation of: glutamate neurotoxicity, inflammation and redox imbalance

2) A pharmacological approach based on a cocktail of drugs is suggested to be the optimal therapeutical strategy (complementary to phototherapy) to protect brain tissue from bilirubin toxicity

A. Bramante,
A. Lorenzon
CBM Scarl, TS

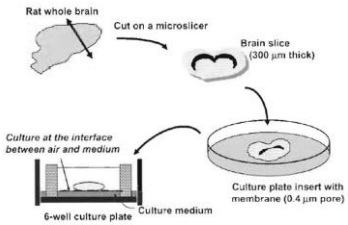
On going *in vivo* trials

Phototherapy is it really so safe?

(therapy)

Phototherapy

(the golden standard treatment for severe neonatal hyperbilirubinemia, generating bilirubin photo-oxidation products): is it really so safe?



The experimental scheme:

1. Hip OBCs exposed to Lumirubin, BoxA and BoxB (The 3 major products of bilirubin photo-oxidation)
2. Viability tests
3. RTqPCR of marker genes

Jana Jasprova,
Libor Vitek,
Charles University
Prague
SPONSOR

Matteo Dal Ben
Silvia Gazzin
Claudio Tiribelli

Phototherapy is it really so safe?

(therapy)

BoxA and BoxB do not exert any effect

Lumirubin exerts a significant pro-inflammatory effect on brain parenchyma

Cautelative warning:

Excessive, not needed phototherapy might be avoided.

Additional study addressing bilirubin photo-derivates bioactivity should be performed

The Brain Organotypic Culture Model of Parkinson Disease

(models / therapy)

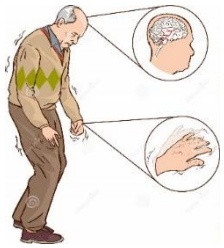
?

Create a PD model in OBCs of Substantia Nigra

Evaluate the (time course of the) mechanisms leading to dopaminergic neurons loss

The experimental scheme:

1. OBCs of Substantia Nigra exposed to Rotenone
2. Viability tests (LDH, counting of dopaminergic neurons number, dendrite length and extension)
3. RTqPCR of marker genes + glutamate



Fondazione Italiana Fegato ONLUS

M. Dal Ben
R. Bongiovanni
S. Gazzin
C. Tiribelli

Ospedali Riuniti di Trieste

R. Moretti, MD PhD
Clinica Neurologica,
Ospedale di Cattinara
SPONSOR

P. Zarattini
Stabulario UniTs

The Brain Organotypic Culture Model of Parkinson Disease

(models / therapy)

Creation of a Parkinson Disease (PD) model in OBCs, mimicking all the features of the human pathology

(dopaminergic neuron loss, alteration genetic markers of PD)

Identification of the early (causative), and late (consequential) molecular events ongoing to dopaminergic neurons loss

Early molecular events may be tested as therapeutical targets for delay (and optimistically stop) PD progression, improving the actual symptomatic therapy

The protective threshold of UCB/Bf

(models)

?

Is bilirubin protective against neurodegenerative disease?

How much low has to be UCB (Bf) to be protective?

The experimental scheme:

1. PD model in OBCs (PD-OBCs), exposed to a full range concentration of free bilirubin
2. Viability tests RTqPCR of marker genes + glutamate

Fondazione Italiana Fegato

M. Dal Ben
S. Tuniz
S. Gazzin
C. Tiribelli

P. Zarattini
Stabulario UniTs

The protective threshold of UCB/Bf

(models)

PRELIMINARY RESULTS

Bilirubin improves PD-OBCs viability

Bilirubin exerts an anti-inflammatory activity on PD-OBCs

Bilirubin exerts an anti-oxidant activity on PD-OBCs

Bilirubin, at very low concentrations (<20 nMol free bilirubin)
seems presenting a protective effect on PD,
by reducing the inflammatory and oxidant status induced by the pathology

2017 Publications





1. *Evaluation of region selective bilirubin-induced brain damage as a basis for a pharmacological treatment.* **Dal Ben**, Bottin, Zanconati, **Tiribelli, Gazzin**. Scientific Report.

2. *Thalamus and language: what do we know from vascular and degenerative pathologies.* Moretti, Caruso, Crisman, **Gazzin**. Neurology India.

3. *Basal ganglia: their role in complex cognitive procedure in experimental models and in clinical practice, a short review.* Moretti, Caruso, Crisman, **Gazzin**. Neurology India.

4. *Homcysteine in neurology: from endothelium to neurodegeneration.* Moretti, **Dal Ben, Gazzin, Tiribelli**. CurrNutrFooDSci.

5. *Vitamin D status, homocysteine and folate in subcortical vascular dementia and Alzheimer dementia: a comparison with old health population.* Moretti, Caruso, **Dal Ben, Conti, Gazzin, Tiribelli**. Frontiers in Aging Neuroscience

6. *Rivastigmine as a sympotmatic treatment for apathy in parkinson's dementia complex: new aspects for this riddle.* **Dal Be**n, Moretti, Caruso. Parkinson's Disease Hindawi .

7. *Frontal Tasks and Behavior in Rigid or Tremor-Dominant Parkinson Disease.* Moretti, Milner, Caruso, **Gazzin**, Rumiati. Am. J. Alzh. Dis. and Other Dem.

8. *Effects of oral administration of silymarin in a juvenile murine model of NALFD/NASH.* **Marin, Gazzin, Gambaro, Dal Ben**, Calligaris, Agnese, Raseni, Avellini, **Giraudi, Tiribelli, Rosso**. Nutrients 2017. Doi:10.3390/nu9091006

9. *Vitamin D in the Brain Small Vessel Disease.* Moretti, Furlanis, Caruso, Signori, **Gazzin, Tiribelli.** Curr. Neurobiol. 2017 8 (3):112-124.





Thanks for your attention

Matteo Dal Ben, Eleonora Vianello, Simone Tuniz, Rosario Bongiovanni, Emanuela Fioriti, Beatrice Anfuso, Caecilia Sukowati, Pablo Giraudi, Devis Pascut, Cristina Bellarosa, Natalia Rosso, Sabrina Corsucci, Lucia Napoli, Sandra Leal, Annalisa Bianco, Emanuele Fioriti, Ana Ruth Araujo, Peter Reyes, Jogy Patrama, Minh Hoang, Vo Van Thanh Niem, Rusdina Bte Ladju, Francesca Gilardi, Adriano del Prete, Claudio Tiribelli

Ospedali Riuniti di Trieste
Azienda Ospedaliero - Universitaria
Servizio Sanitario Regionale

C. Bottin
F. Zanconati
T. Marcuzzo
Dip. Anatomia Patologica
Ospedale di Cattinara
Trieste, It

Ospedali Riuniti di Trieste
Azienda Ospedaliero - Universitaria
Servizio Sanitario Regionale

R. Moretti, MD PhD
Clinica Neurologica,
Ospedale di Cattinara
Triese, It

Azienda Ospedaliero Universitaria
Santa Maria della Misericordia di Udine

A. Dardis
S. Zampieri
Dip. Malattie Rare
S. Maria della Misericordia
Udine, It

P. Ostano
Fondazione Edo ed Elvo
Tempia Valenta
Università degli Studi di
Torino, It

cbm
cluster in biomedicine

A. Bramante
A. Lorenzon
CBM Scarl
Trieste, It

P. Zarattini
Stabulario UniTs,
Trieste, It

Jana Jasprova,
Libor Vitek,
Charles University
Prague, CZ

CRNL
Centre de Recherche
Neurosciences Lyon

N. Strazielle,
J-F Ghearsi-Egea,
Centre de Recherche en Neurosciences
U1028 / UMR 5292
Lyon, Fr