AUTOPHAGY GOOD AND BAD: A GENUINE TARGET FOR RUBBISH REMOVAL IN NEUROPATHOLOGIES?

Philip M. Beart,
Florey Institute of Neuroscience & Mental Health,
University of Melbourne,
Parkville, VIC 3010, AUSTRALIA.
Patents for glutamate reuptake modulators

Blockade by polyamine NMDA antagonists related to ifenprodil of NMDA-induced synthesis of cyclic GMP, increases in calcium and cytotoxicity in cultured neurones

(Philip M. Beart, *Arne Schousboe & *Aase Frandsen

Delayed Treatment With AM-36, a Novel Neuroprotective Agent, Reduces Neuronal Damage After Endothelin-1-Induced Middle Cerebral Artery Occlusion in Conscious Rats

Jennifer K. Callaway, PhD; Melissa J. Knight, BSc; Diane J. Watkins, BSc; Philip M. Beart, DSc; Bevyn Jarrott, PhD

Multiple patents – UK company collapsed

Vampire Bat Salivary Plasminogen Activator (Desmoteplase) Inhibits Tissue-Type Plasminogen Activator-Induced Potentiation of Excitotoxic Injury

Courteny Reddop, BSc (Hons); Randal X. Moldrich, PhD; Philip M. Beart, DSc; Mark Faro, BSc (Hons); Gabriel T. Libaireau, PhD; David W. Howells, PhD; Karl-Uwe Petersen, MD; Wol-Dieter Schieling, MD, PhD; Robert L. Medcalf, PhD

Lundbeck discontinued Dec 2014

Transcriptomic Profiling of Astrocytes Treated With the Rho Kinase Inhibitor Fasudil Reveals Cytoskeletal and Pro-Survival Responses

CHEW L. LAU,* VICTORIA M. PERREAU,2,3 MINGHUI J. CHEN,4 HOLLY S. CAT,1,2 DANIEL MERLO,1,2 NAM S. CHEUNG,6,6 ROSS D. O’SHEA,1,6 AND PHILIP M. BEART7

Patented as anti-inflammatory ALS/MND
## DISEASE BURDEN – UNMET THERAPEUTIC NEED

<table>
<thead>
<tr>
<th>PATHOLOGY</th>
<th>INCIDENCE</th>
<th>COSTS PER ANNUM</th>
</tr>
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<tbody>
<tr>
<td>Stroke</td>
<td>50,000 per annum</td>
<td>$2 billion</td>
</tr>
<tr>
<td>Perinatal hypoxia</td>
<td>1-4 per 1000 infants</td>
<td>$3 billion (USA)</td>
</tr>
<tr>
<td>Motor Neuron Disease</td>
<td>1300, 532 died (2006)</td>
<td>Impacts carers, families &amp; friends</td>
</tr>
<tr>
<td>Parkinson’s Disease</td>
<td>30-300 per 10,000</td>
<td>$500 million</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>1 per 10,000</td>
<td>??</td>
</tr>
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### Disease Apoptosis, Autophagy, Prog Necrosis

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>APOPTOSIS</th>
<th>AUTOPHAGY</th>
<th>PROG NECROSIS</th>
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<tr>
<td>Stroke</td>
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<td>✔</td>
<td>✔</td>
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<tr>
<td>Parkinson’s</td>
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<tr>
<td>MND/ALS</td>
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<tr>
<td>Huntington’s</td>
<td>✔</td>
<td>✔</td>
<td>✔?</td>
</tr>
</tbody>
</table>
Pathobiology of ischaemic stroke: an integrated view

Ulrich Dirnagl, Costantino Iadecola and Michael A. Moskowitz
Micromolar L-glutamate induces extensive apoptosis in an apoptotic-necrotic continuum of insult-dependent, excitotoxic injury in cultured cortical neurones

Nam S. Cheung, Catherine J. Pascoe, Sarah F. Giardina, Christopher A. John, Philip M. Beart *
DEATH IS NOT SIMPLE

Figure 1. Necrosis predominates in the ischemic core, whereas apoptosis and autophagy are often observed in the penumbra after focal ischemia. In the ischemic core (A) necrosis refers to morphological signs seen after a cell has already died and reached equilibrium with its surroundings (E). The presence of necrosis tells that a cell has died but not necessarily how death occurred. In the penumbra, hybrid forms of cell death occur.

Rami & Kogel,
Autophagy (2008)
EDITORIAL HIGHLIGHT

Autophagy: a common road to perdition in acute brain injuries and Alzheimer's disease

Giuseppina Tesco
Alzheimer’s Disease Research Laboratory, Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts, USA

ORIGINAL ARTICLE

Autophagosomes accumulation is associated with β-amyloid deposits and secondary damage in the thalamus after focal cortical infarction in hypertensive rats

Jian Zhang,*† Yusheng Zhang,† Jingjing Li,† Shihui Xing,* Chuo Li,* Yiliang Li,* Chao Dang,* Yuhua Fan,* Jian Yu,* Zhong Pei* and Jinsheng Zeng*
Autophagy

- **Cellular homeostasis:** Autophagy recycles cellular damaged components & responds to energy deficits
- **Cellular defence:** Autophagy can help cells avoid damage and death under various stresses
Overview of the General Autophagy Pathway

**PREINITIATION COMPLEX**

- mTOR
  - Amino acid and serum starvation
- AMPK
  - Low energy
  - Glucose deprivation
- ULK1/2
  - ATG13
  - FIP200
  - Other signals

**INITIATION COMPLEX**

- Class III PI3K Complex
  - Bcl-2
  - Beclin 1
  - ATG14L
  - VPS34
  - VPS15

**ELONGATION REACTION**

- Ubiquitin-like protein conjugation systems
  - ATG7
  - ATG12
  - ATG10
  - ATG5
  - ATG6
  - ATG16L1
  - ATG4

**Membrane Source**

- ER exit site, ER/mitochondria contact site, mitochondria, Golgi apparatus, ERGIC, recycling endosome, plasma membrane

**Degradation and recycling**

- Physiological functions
  - Nutrient and energy homeostasis
  - Removal of damaged/unwanted organelles
  - Removal of aggregate-prone proteins
  - Removal of intracellular pathogens

- Vesicle completion

- Autophagosome

- Fusion

- Late endosome or MVB

- Lysosome

Autophagy is generally good for cells

Autophagic cell death (ACD)
Autophagy

- **Cellular homeostasis**: Autophagy recycles cellular damaged components & responds to energy deficits
- **Cellular defence**: Autophagy can help cells avoid damage and death under various stresses
- **Cell death process (PCD Type II)**: Autophagy directly contributes to the cell death outcome
Diversity of cell death in neurons

**Oxidative stress**

**Caspase-independent death**

Nagley et al. Beart. BBA 2010
Conversion of LC3-I to LC3-II after treatment with STS or H$_2$O$_2$ indicates an increase in autophagic activity.

Higgins et al. CMLS 2011

Autophagy is induced in these neurons treated with either H$_2$O$_2$ or STS.
The progression to cell death is blocked by knockdown of Atg7 in neurons treated with H$_2$O$_2$ but **NOT** with STS.

Higgins et al. CMLS 2011
Death outcomes differ in terms of biochemical mechanisms and cellular morphologies.

Relative involvement of individual death processes depends upon the neuronal type and stressor (Nagley et al., 2010).

Apoptosis and autophagy. Death has characteristics of apoptosis (PCD-Type I), but inhibition of autophagy fails to block death – the events are not functionally linked.
What about autophagy & death following sustained exposure to oxidative stress?
The Approach: Cortical neurons treated with superoxide (O$_2^-$) generated from xanthine/xanthine oxidase and catalase (XXC) alongside a reference apoptotic inducer staurosporine (STS).
Cell death by $O_2^-$ flux is transiently blocked by Atg7 and Endo G knockdown

Atg7 knockdown

EndoG knockdown

Transient autophagic cell death

Transient Programmed necrosis

Cell death still occurs, suggesting $O_2^-$ overwhelms cells to undergo unregulated necrosis
ACD overwhelmed by unregulated necrosis

- Caspase activity in the presence of oxidative stress has been reported in other neuronal cell systems, BUT it is clear that this is not the case under our experimental conditions (i.e. no apoptosis).
- Early dissipation of ΔΨm followed by rapid redistribution of cyt c, Smac/DIABLO and Endo G indicated mitochondrial involvement.
- Both autophagic cell death and programmed necrosis are activated.
- We envisage that pathways leading to autophagic death and programmed necrosis may be running in parallel in early stages.
- Complete blockade of cell death was not achieved by such disruption of either ACD or programmed necrosis, suggesting that ultimately cells default to death by unregulated necrosis.
A role for **autophagy** in ecstasy (MDMA)-induced death of serotonin neurones?

MDMA-induced brain injury

- Slow, PCD with caspase-dependent component?
- Effects include ox stress, DNA damage, inflammation
- Ubiquitinated inclusions
- Disturbed energy metabolism
- Impaired axonal transport = damaged organelles?
- MDMA induces Atg5 expression in cell lines
- UPS recruited (autophagy?)

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**Graph:**

- **Y-axis:** Fold-increase of LC3-II/LC3-I normalised to actin
- **X-axis:** Time (h) 24, 48, 72
- **Legend:**
  - Ctrl
  - 100 uM

**Bars:**

- 24h: Ctrl - 0, 100 uM - 0
- 48h: Ctrl - 0, 100 uM - continue
- 72h: Ctrl - 0, 100 uM - increase
Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington’s disease

Claudia Rose¹, Fiona M. Menzies¹, Maurizio Renna¹, Abraham Acevedo-Arozena², Silvia Corrochano², Oana Sadiq¹, Steve D. Brown² and David C. Rubinsztein¹,*

¹Department of Medical Genetics, University of Cambridge, Cambridge Institute for Medical Research, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0XY, UK and ²Medical Research Council Mammalian Genetics Unit, Harwell, Oxfordshire, UK

Received December 11, 2009; Revised February 3, 2010; Accepted February 25, 2010

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disease caused by a polyglutamine expansion in huntingtin. There are no treatments that are known to slow the neurodegeneration caused by this mutation. Mutant huntingtin causes disease via a toxic gain-of-function mechanism and has the propensity to aggregate and form intraneuronal inclusions. One therapeutic approach for HD is to enhance the degradation of the mutant protein. We have shown that this can be achieved by upregulating autophagy, using the drug rapamycin. In order to find safer ways of inducing autophagy for clinical purposes, we previously screened United States Food and Drug Administration-approved drugs for their autophagy-stimulating potential. This screen suggested that rilmenidine, a well-tolerated, safe, centrally acting anti-hypertensive drug, could induce autophagy in cell culture via a pathway that was independent of the mammalian target of rapamycin. Here we have shown that rilmenidine induces autophagy in mice and in primary neuronal culture. Rilmenidine administration attenuated the signs of disease in a HD mouse model and reduced levels of the mutant huntingtin fragment. As rilmenidine has a long safety record and is designed for chronic use, our data suggests that it should be considered for the treatment of HD and related conditions.
Activation of Autophagy: Neuroprotection

Murine serotonin (5-HT) neurones in culture
Autophagy: a valid clinical target in MND/ALS?

Suppression of mutant SOD1 aggregation stimulates autophagy in NSC-34 MN cell line

*Autophagy activator provided by Servier*
Fig. 2. One hypothetical model where the extent of autophagy dictates the fate of neurons under stress. When faced with a harsh stress, neurons rely on autophagy induction as a means of protection and damage limitation, suppressing cell death and promoting survival. This means an inefficient or inhibited level of autophagy can be detrimental to neuronal health. At the other end of the spectrum, an excessive autophagic response may result in the degradation of vital cellular components, culminating in ACD. Therefore, it seems that in order for autophagy to exert its protective effects, a balance needs to be maintained to avoid neuronal death.
What is Mitophagy???

- A form of autophagy where damaged or dysfunctional mitochondria selectively undergo degradation, can occur as a consequence of PCD when mitochondria fragment and remodel inner-membrane cristae.

- Moreover, cellular bioenergetics is entwined with mitochondrial dynamics, and mitochondrial insults, including depolarization and ETC inhibition, trigger mitochondrial fragmentation.
ACTIVATE AUTOPHAGY IN YOUR MODEL

Neuroprotection of kaempferol by autophagy in models of rotenone-mediated acute toxicity: possible implications for Parkinson’s disease

Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington’s disease

Tsc1 (hamartin) confers neuroprotection against ischemia by inducing autophagy

Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43
<table>
<thead>
<tr>
<th>Strategy</th>
<th>Neurodegenerative Disease</th>
<th>Changes to Pathology</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Pharmacological</td>
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<tr>
<td>Rapamycin</td>
<td>Alzheimer’s disease</td>
<td>Autophagy induction; reductions in Aβ and cognitive recovery in AD mice</td>
<td>[71],[112]</td>
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<tr>
<td></td>
<td>Huntington’s disease</td>
<td>Reductions in Htt aggregate formation, improvements in behavioral tests in mice</td>
<td>[111]</td>
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<td>Parkinson’s disease</td>
<td>Reductions in α-synuclein accumulation, alleviation of neurodegenerative behavior in mice</td>
<td>[113],[114]</td>
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<td>Autophagy induction; enhanced clearance of mutant Htt, improved motor performance in mice</td>
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<td>Autophagy induction and increased flux; removal of Aβ oligomers, cognitive improvements in mice</td>
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<td>Glucosylceramide inhibitors</td>
<td>Niemann-Pick Type-C 1</td>
<td>Correction of autophagic flux; improved clearance of cholesterol and autophagic vesicles in mouse and cat models, prolonged neuron survival</td>
<td>[119],[120]</td>
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<td>Upregulation of lysosomal and autophagy genes;</td>
<td>[124]</td>
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<td>enhanced clearance of tau, α-synuclein, and mutant Htt aggregates</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>Parkinson’s disease</td>
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<td>[67]</td>
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**Figure 3** | mTOR-independent autophagy induction pathways. Chemical screens for new autophagy-inducing agents have identified the cyclical Ca²⁺-calpain-Goα and cAMP-Epac-PLC-ε-IP₃ pathways as pharmacologically tractable for the modulation of autophagy. Inhibition of various components of these pathways results in autophagy induction. However, the precise mechanism by which levels of cAMP, Ca²⁺, calpain, inositol or IP₃ control autophagy have yet to be elucidated.
AUTOPHAGY IN HUMAN NEUROPATHOLOGIES?

Autophagy is increased in mice after traumatic brain injury and is detectable in human brain after trauma and critical illness

Robert S.B. Clark, Hülya Bayir, Charleen T. Chu, Sean M. Alber, Patrick M. Kochanek & Simon C. Watkins

Neuropathological role of PI3K/Akt/mTOR axis in Down syndrome brain

Marzia Perluigi a,1, Gilda Pupo a,1, Antonella Tramutola a, Chiara Cini a, Raffaella Coccia a, Eugenio Barone a, Elizabeth Head b, D. Allan Butterfield b,c, Fabio Di Domenico a,*

Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnestic mild cognitive impairment and late-stage AD

Antonella Tramutola,† Judy C. Triplett,† Fabio Di Domenico,† Dana M. Niedowicz,‡ Michael P. Murphy,‡ Raffaella Coccia,§ Marzia Perluigi* and D. Allan Butterfield†‡
MCI = mild cognitive impairment
AD = late Alzheimer’s disease
PCAD = pre-clinical AD
**Autophagy, mitophagy & UPS in vivo stroke injury**

Immunoblotting of contralateral (Contra) and ipsilateral (Ipsi) hemisphere tissue, cortex (COR) and striatum (STR), of a mouse brain after 1 h occlusion & 24 h reperfusion.

Samples probed for LC3 (A), PINK1 (C) & p62 (D). B. Samples probed for Ub.

Enhanced expression in cortex and striatum of the ipsilateral hemisphere highlighting increased autophagy flux & possible mitophagy. This pattern is mirrored by Ub labelling (B), revealing increased protein ubiquitination. Loading control: β-actin (Act).
1,026 Experimental Treatments in Acute Stroke

Tested in vitro but not in vivo

Tested in vivo did not improve outcome

Improve outcome in vivo but not taken forward to clinical trial

Improved outcome in vitro and in vivo but did not improve outcome in clinical trial

No in vitro or in vivo development history

Dedicated Stroke Units
Aspirin
Decompression (Malignant MCAo only)

Tissue plasminogen activator

1,026
423
229
96
15
1
3
What we are talking about today matters to human brain health

Cortex of patients with history of stroke

Autophagic marker LC3 in human stroke brain.

Peter Crack, Catriona Maclean, Philip Beart, Tony Frugier
Unpublished observations, manuscript in revision
EVIDENCE FOR THE RECRUITMENT OF AUTOPHAGIC VESICLES IN HUMAN BRAIN AFTER STROKE

SQSTM1/ p62

Fold change in mRNA levels

Control (n=4) Infarct (n=4)

* p62 immunolabelling in stroke injury
Parkin-mediated mitophagy

Ubiquitination

PINK1, Parkin

Transport and clustering

HDAC6

Autophagosome formation

Ub-PROTEIN

ATG8 HOMOLOG

CARGO RECEPTOR

Ub E3 LIGASE

NH₂ → PB1 → ZZ → TBS → UBA → COOH

NLS1

NLS2

NLS3

NLS4

NLS5

PEST

LIR

KIR

+ NF-κB activation
+ Neuronal survival
+ Energy homeostasis
+ Mitophagy

Rpt1, αPKCs, MEKK3, ERK1

RIP1, AMPA

TRAF6

Autophagic clearance
• NF-κB signaling
• IL-1 signaling
• RANK signaling

LIR KIR

Proteasomal degradation
• Protein aggregation

Inhibition of p62 promoter by oxidative stress

p62 expression

Epigenetic regulation

Binding of p62 to tangles and other protein aggregates

Defects in neuronal signaling

Decreased protein trafficking and autophagic clearance

AD pathology
Abnormal protein accumulation
Synaptic defects and neurodegeneration
Memory loss

Fig. 3. Schematic scheme for the functional role of p62 in the accumulation of damaged proteins.
Conclusions and overview

• Paucity of work studying autophagy/mitophagy in neurons (which do not behave like immortalised cancer cells, most often studied in this field)

• Recruitment of autophagy after neuronal injury is complex

• ACD exists – is energy balance the determinant?

• “Good” autophagy that maintains a healthy cell AND can rescue the cell after some stresses ..... *versus* “bad autophagy” that can eventually kill the cell

• “Good” autophagy (= non-ACD) valid pharmacological target