RESEARCH ARTICLE

Dictyostelium discoideum, strain AX2, a novel model system for studying Autophagy

Kumar Sree S, Thanawalla Ayesha, and Pote Archana

Department of Life Sciences, Sophia College, Bhulabhai Desai Road, Mumbai, 400026

Manuscript details:	ABSTRACT
Available online on http://www.ijlsci.in ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print) Editor: Dr. Arvind Chavhan Cite this article as: Kumar Sree S, Thanawalla Ayesha, and Pote Archana (2015) Dictyostelium discoideum, strain AX2, a novel model system for studying Autophagy, Int. J. of Life Sciences, Special Issue, A5: 55-59. Acknowledgement: The authors would like to thank Principal, Sophia College and Department of Life Sciences for facilities extended We	Dictyostelium is an eukaryote, a protist, and a slime mold. Dictyostelium shows developmental vacuolated cell death (autophagy) in the stalk. Macroautophagy is an intracellular degradative process for cytosolic components at the lysosome. Derailing of this mechanism is implicated in several neurodegenerative diseases, as it leads to accumulation of mutant aggregate proteins. Dictyostelium lacks the required machinery for apoptosis, and undergoes cell death only by autophagy or necrosis. Starvation in Dictyostelium sets in the mode of multicellular development in which stalk cells enlarge and altruistically die by autophagy which can be mimicked in vitro. In the present work, Lithium and Valproic acid were used to observe autophagy in the AX2 strain. Monolayer cultures of AX2 strain of Dictyostelium was standardized to observe the percentage of vacuolated autophagic cells using microscopic and staining techniques. Our studies established that AX2 strain can be a new model system to study autophagic cell death in neurodegeneration and can be used as a pharmacological model for putative drugs as well. Keywords : Macroautophagy, AX2 strain, Monolayer cultures, Dictyostelium
are grateful to UGC Minor Research grant for financial support of our research Copyright: © Author, This is an open access article under	INTRODUCTION Macroautophagy is an intracellular lysosomal degradative pathway. It
the terms of the Creative Commons Attribution-Non- Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non- commercial and no modifications or adaptations are made	is implicated in neurodegenerative states as a major mechanism in the clearance of mutant aggregate proteins and as a mechanism of neuronal cell death (Hochfeld <i>et al.</i> , 2013). So far apoptosis and necrosis have been given much attention but macroautophagy has not been well probed for its role in cell death and neuronal damage. In mammalian systems, the study of its mechanism, is often complicated by interfering apoptotic machinery.

discoideum. A simple eukaryote, which goes through two stages during

its lifetime; a unicellular amoeboid stage and a multicellular fruiting body, consisting of stalk cells and spore cells. The formation of stalk cells is in part achieved by macroautophagy (henceforth referred to as autophagy) (Otto *et al.*, 2003). The signals required for autophagic cell death are starvation, cAMP (cyclic AMP) and DIF1 (differentiation inducing factor 1). The major advantage of the system is that it lacks apoptotic machinery, thus making the system ideal for the study of autophagic cell death (Giusti *et al.*, 2008).

The present study has used this simple model system to study autophagy and the effects of the neuroprotective agents Lithium and Valproic acid on it. The stalk length was measured to infer the changes in autophagic process brought about by these agents. The formation of autophagic stalk cells is also studied by inducing vacuolisation invitro in monolayers. By observing the changes in cell morphology as compared to healthy controls and induced autophagic cells, the effect of Lithium and Valproic acid on autophagy may be inferred.

MATERIALS AND METHODS:

Maintenance

D.discoideum AX2 vegetative cells, courtesy [Dr Malik's Lab, TIFR (Mumbai)] were grown in flask, suspended in liquid HL5 media (Ashworth and Watts, 1970) Media and Buffers; HL5 media, KK2 buffer, Sorensons buffer (SB). Reagents; 3 mM cAMP (cyclic AMP; 3',5'adenosine cyclic monophosphate, sodium salt, Sigma A6885), 100 nM DIF-1 {DIF-1: differentiation-inducing-factor 1,1-(3,5-dichloro-2, 6-dihydroxy- 4 – methoxyphenyl) - hexan-1-one; DN1000, Affiniti Research Products, Exeter, UK } , LiCl2 (Lithium chloride), VPA (Valproic acid).

In vivo Stalk length measurement:

For stalk length measurements *D.discoideum* AX2 spores were collected from fruiting bodies growing axenically on SM agar (Sussman and Sussman, 1967). Approximately 15-20 spores

were suspended in KK2 buffer supplemented with 2mM, 5mM and 10mM LiCl2 and were placed on SM agar plates. Controls were maintained by suspending the spores in KK2 buffer. Plates were incubated at 22°C in BOD incubator for 5 days. Sets were run in duplicates, imaged and the stalk length measured by Image J software [40X Axioscope].

Stalk cell differentiation in vitro:

For inducing stalk cell differentiation in monolayers (Fey *et al.*, 2007) 30µl overnight culture of *D. Discoideum*-AX2 (Axenic) cells in liquid HL5 media (cell count 3x105/ml) were placed on the coverslip and allowed to adhere for 24 hours. Media was removed and the cells were washed with Sorensons Buffer (SB buffer), chemical treatments were then added to the cells for 24hours: SB buffer, 3mM cAMP, 10mM lithium chloride, 10mM VPA. Cells were then washed in SB buffer, fixed in 4% pfa for 20mins, mounted in glycerol and observed using phase contrast and confocal imaging. The percentage of vacuolated cells was calculated.

RESULTS AND DISCUSSION

At organismal level: (in vivo conditions):

Our results suggest that lithium and VPA enhances the stalk length of the fruiting bodies (Fig.1& 2). *Dictyostelium* stalk cells are dead by autophagy, adding lithium and VPA caused an increase in length of the stalk, which could mean the number of cells being differentiated into stalk cells increases. These increased proportions of stalk cells indicate more number of cells undergoing autophagic cell death on treatment with lithium.

Lithium's inhibition on the spore cell differentiation is mimicked by a mutation in the gene gskA which encodes a homologue of the signaling molecule glycogen synthase kinase 3 (GSK-3). These molecules are conserved signaling molecules mediated in various GSK-3 pathways. The neuroprotective effects of lithium are

56 | NCFDTA, 2015

mediated, at least in part, by the inhibition of GSK-3 β activity in neurons (Diniz *et al.*, 2013). Hence studying the pathways mediated by GSK-3 in a simple model might help in elucidating the possible targets of lithium on these pathways.

Autophagy was successfully induced in vitro:

There was increase in autophagic activity in cells treated with cAMP as compared to the control (Fig.3). Despite the fact that cAMP is a natural chemoattractant and facilitates attraction of the cells during starvation, intracellular cAMP has been shown to induce autophagy via recruiting components of the cell cycle. In order to become autophagic, Dictyostelium cells require two stimuli a combination of starvation and cAMP. Cells treated with cAMP were compared with unstarved cells and cells which were only starved (not treated with cAMP). The former showed a greater percentage of cells with autophagic vacuoles. Starvation was used as an initial stimulus to successfully induce autophagy induction in the monolayer of Dictyostelium discoideum AX2 cells. Thus the conditions present in vivo due to starvation secretion of cAMP were successfully mimicked in vitro inducing autophagy in the monolayer culture.

Lithium induces macroautophagy in *D. Discoideum*.

Dictyostelium cells treated with Lithium showed vacuolation comparable to that of the cAMP

treated positive control. On treatment with 5mM Lithium, AX2 cells showed an increase in the number of cells showing vacuoles comparable to cyclic AMP treated cells. Furthermore, cAMP and lithium when added together, greatly enhanced vacuolation showing an additive effect (Fig. 3). Lithium acts by competing with the cofactor Mg 2+, thus inhibiting intracellular enzymes. It induces autophagy by inhibiting the enzyme inositol monophosphatase (Sarkar et al., 2005). Lithium has been shown to induce autophagy by inhibiting the enzyme inositol monophosphatase. This inhibition causes a depletion of intracellular inositol triphosphate (IP3) which has been shown to induce autophagy and protein clearance (Sarkar et al. 2005). By inducing the formation of autophagic vacuoles in Dictyostelium we have shown that even in a simple system Lithium is a potent inducer of autophagy. In order to check whether Lithium had the ability to enhance autophagy even in the presence of cAMP, 5mM Lithium was added along with cAMP. Results indicate a large increase in the number of cells vacuolated showing that Lithium enhances autophagy in a monolayer of *Dictyostelium* cells. In Dictyostelium, DIF1 induces autophagic cell death and this activity has been found to be mediated by the IP3R, i.e. the receptor and calcium fluxes are also important for cell death signalling (Ganley et al., 2009). Thus inhibition of the IP3R by lithium could prevent autophagic cell death.



Fig.1&2: Figures 5 and 6 show quantitative data of the increase in stalk length with both Lithium and Valproic acid. Fig.1with Lithium, Fig.2 with Valproic acid



Fig. 3: Graph of percentage vacuolated cells. Lithium and cAMP show comparable results, Li and cAMP have a synergistic effect. **indicates p<0.005

Although poles apart both mechanisms involving the IP3R point to an important role in autophagy and autophagic cell death, in which intervention with lithium could lead to cell protection. Thus the timing of intervention with lithium could be an interesting way to either enhance autophagy as well as a method to prevent cell death. Further, lithium's protection in mammalian cells which do not use DIF would be interesting to investigate.

Valproic acid enhances autophagy:

On treating the cells with 5mM VPA a significant increase in autophagic vacuoles was observed as compared to the control (Fig.3). However when added together, vacuolization was shown to be decreased. Theoretically this can be postulated to be caused by a decrease in the intracellular levels of IP3 and an upregulation in the Erk 1/2pathway due to increase in ROS (Cuervo and Dice, 2000). The Erk 1 (extracellular-signal-regulated kinases) pathways has been shown to decrease IP3 levels via the disheveled protein in the noncanonical Wnt-1 pathway (Ding, & Shen, 2008). This will further lead to an increase in autophagy. The increase in IP3 levels could lead to an increase in Ca2+ levels in the cell which will lead to an upregulation in autophagy. cAMP has also been shown to increase intracellular cAMP levels. Thus if the upregulation of Erk 1/2 leading to a decrease in IP3 levels is countered with an increase in IP3 levels due to cAMP expression then at a particular concentration range the net change in IP3 levels is zero/negligible. This would lead to no change in autophagy induction in the cells. Thus autophagy may occur at higher (as seen in our *in vivo* results) and lower concentrations of VPA but will not lead to a significant increase in autophagy within a certain concentration range. However, this needs to be confirmed by a systematic study of the cellular and molecular effects of valproic acid in *D. discoideum*.

CONCLUSION

In most neurodegenerative diseases where autophagy becomes dysfunctional, upregulating the process of autophagy to degrade the aberrant proteins would be a potential therapeutic. However, the complex roles of autophagy in survival and death should be considered when designing pharmacological therapeutics for disease. Here we have used lithium and valproic acid to test its effect on the process of autophagy in a simple model. The molecular markers involved in autophagy and autophagic cell death would help us understand the switch from autophagy for survival to a mechanism of death. Hence *Dictvostelium* may prove to be a beneficial model to test the effect of neuroprotective drugs in enhancing autophagy and protecting the cell from autophagic cell death and could serve as primary screen to study the effect of pharmacological inducers on autophagy and autophagic cell death.

REFERENCES

- Ashworth JM and Watts DJ (1970) Metabolism of the cellular slime mould Dictyostelium discoideum grown in axenic culture. *Biochemical Journal*. 119(2):175–182.
- Cuervo AM and Dice JF (2000) Regulation of Lamp2a Levels in the Lysosomal Membrane. *Traffic*, *1*, 570–583.

- Ding M and Shen K (2008) The role of the ubiquitin proteasome system in synapse remodeling and neurodegenerative diseases. *BioEssays : News and Reviews in Molecular, Cellular and Developmental Biology, 30*(11-12), 1075–1083.
- Diniz BS, Vieira R, Forlenza OC (2013) Lithium and neuroprotection: translational evidence and implications for the treatment of neuropsychiatric disorders. *Neuropsychiatric Disease Treatment*. 9, 493–500.
- Fey P, Kowal AS, Gaudet P, Pilcher KE, Chisholm RL (2007) 'Protocols for growth and development of *Dictyostelium discoideum*. *Nature Protocols* 2:1307-16.
- Fuchs Y and Steller H (2011) Programmed cell death in animal development and disease. *Cell*, 147(4), 742-58.
- Ganley IG, Lam DH, Wang J, Ding X, Chen S, and Jiang X (2009) ULK1·ATG13·FIP200 Complex Mediates mTOR Signaling and Is Essential for Autophagy. *The Journal of Biological Chemistry*, 284(18), 12297–12305.
- Giusti C, Kosta A, Lam D, Tresse E, Luciani M-F, and Golstein P (2008) Analysis of Autophagic and Necrotic Cell Death in Dictyostelium. Methods in Enzymology, Elsevier 446:1-15

- Hochfeld WE, Lee S and Rubinsztein DC (2013) Therapeuticinduction of autophagy to modulate neurodegenerative diseaseprogression.Acta Pharmacol. Sin.34, 600–604.
- Otto GP, Mary YW, Kazgan N, Anderson OR, Kessin R (2003) Macroautophagy is Required for Multicellular Development of the Social Amoeba Dictyostelium discoideum. *The Journal of Biological Chemistry*. 278(20), 17636–17645.
- Sarkar Sovan Andres, Floto R Zdenek, Berger Sara Imarisio, Axelle Cordenier, Matthieu Pasco, Lynnette J Cook and David C Rubinsztein (2005) 'Lithium induces autophagy by inhibiting inositol monophosphatase' *Journal of Cell Biology* 170(7),1101–1111.
- Sussman M and Sussman R (1967) Cultivation of Dictyostelium discoideum in axenic mediumBiochemical and Biophysical Research Communications 29(1), 53-55
- Warren E Hochfeld, Shirley Lee, David C Rubinsztein (2013) Therapeutic induction of autophagy to modulate neurodegenerative disease progression. Acta Pharmacologica Sinica. 34, 600–604.

© 2015 | Published by IJLSCI