Novel pro-longevity compounds for the treatment of neurodegenerative diseases

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Summary

The ageing of the population has created an urgent need to develop approaches targeting the ageing process. In model organisms, the process of ageing can be manipulated by both genetic manipulations and dietary interventions. Most notably, caloric restriction (CR), a reduction in calorie intake without malnutrition, extends longevity and retards age-related diseases, including neurodegeneration. Developing CR mimetics, compounds that reproduce the health and longevity benefits of CR without its side-effects, is therefore of widespread medical and commercial interest.

Using a network pharmacology approach applied to publicly-available drug gene expression data, we have recently identified 11 novel candidate CR mimetics. Five of these have already been tested in the roundworm *C. elegans*, an established model of ageing and of CR, with four significantly extending lifespan. In order to advance in the translational pathway, it is essential that we now demonstrate that these compounds can be applied to specific diseases. In this project, we aim to study the 11 new candidate CR mimetics for lifespan and healthspan effects in two worm models of neurodegenerative diseases: a *dnj-14* null mutant model of adult-onset neuronal ceroid lipofuscinosis and a worm model of frontotemporal dementia expressing a human Tau mutant, parkinsonism-17.

There is considerable current interest in repurposing drugs for the treatment of neurodegenerative conditions. We expect to identify new drugs with potential for the treatment of neurodegenerative diseases that we can then take on to further and longer studies in mammalian models. Though additional studies will be necessary beyond this project, this work can culminate in the development of new treatments for neurodegenerative diseases.

RESEARCH PROPOSAL

1) Background to the project

Ageing is a major biological process with a profound impact on human society and modern medicine. According to projections from the Office for National Statistics & Government Actuary Department, by 2050 the proportion of people over 60 in Britain will rise from 21% to almost 40%. This “greying” of the population is arguably the major biological and biomedical challenge of the 21st century not only for the UK but for other industrialized countries as well since the incidence of age-related diseases, and neurodegenerative diseases in particular, is expected to increase dramatically in the coming decades.

Brain ageing frequently underlies cognitive decline and is a major risk factor for neurodegenerative conditions such as Alzheimer's disease (AD) and Parkinson's disease (PD) for which there is no adequate treatment. Mental health is also a major concern of ageing adults. The ageing of the population means there is a great and urgent unmet need to develop approaches and therapies targeting the ageing process and capable of ameliorating age-related neurodegenerative diseases [1].

In model organisms, the process of ageing can be manipulated by both genetic manipulations and dietary interventions. Most notably, caloric restriction (CR), a reduction in calorie intake without malnutrition, has consistently been shown to increase longevity and preserve health in several model organisms, including yeast, flies, worms and rodents. This makes CR a central paradigm to study dietary modulation of ageing and health. In mice and rats, CR can extend longevity by up to 50%, delay physiological ageing and postpone or diminish the morbidity of most age-related diseases [2]. CR can also delay brain ageing and prevent neurodegeneration [3, 4]. Ongoing studies in rhesus monkeys [5, 6] suggest that CR has health benefits, and one study found a marked reduction in mortality in monkeys under CR [5]. These studies indicate that the health benefits of CR are likely to extend to humans. Indeed, CR induces changes in humans that are associated with healthy ageing and protection from age-related pathologies [2]. However,

DR has multiple negative side-effects [7], and therefore developing DR mimetics (drugs or foods that reproduce the actions of DR without its side-effects) is of widespread interest [8].

2) Preliminary results leading to this application

Network pharmacology approach: To identify new candidate CR mimetic compounds, we cross-linked mammalian molecular signatures of CR with drug signatures available in the Connectivity Map [9]. We discovered 11 small molecules that significantly (after Benjamini correction) induce gene expression changes overlapping with those occurring in CR [9]. Molecules include rapamycin, which was our top hit and extends lifespan from yeast to mice [8, 10], and wortmannin and LY294002 that also extend lifespan in invertebrates and inhibit the PI3K pathway, which is closely associated with ageing via insulin/IGF1 signalling [11]. Compounds not yet associated with ageing were also identified, like allantoin. The fact that rapamycin was the top result among over 1,000 compounds tested is a strong indicator that this approach provides biologically-relevant results. Therefore, these 11 compounds represent promising candidates to act as CR mimetics. Five of our 11 candidates have already been tested in the roundworm *C. elegans*, an established model of ageing and of CR, with four significantly extending lifespan (see [9] and below). In order to advance in the translational pathway, however, it is essential that we now demonstrate that these compounds can be applied to specific diseases.

Experimental validation in worms: With funding from the Royal Society we established worm work in my lab. We obtained *eat-2* mutants (DA465 strain), which are an established long-lived strain that is also a genetic model of CR because animals have altered pharyngeal function and are unable to ingest as much food as N2 wild-type controls [12]. We then performed a lifespan analysis using standard conditions [13] to test rapamycin for life-extending effects. Our results reveal a clear, statistically significant (Log-rank test) life-extension in N2 controls (Figure 1A), the effects of which are not observed in *eat-2* mutants, demonstrating that rapamycin is indeed a CR mimetic. Moreover, the observed results (~20% life-extension) are similar to those published by other labs [14]. In addition, we further tested 4 other candidate compounds, 3 of which (allantoin, trichostatin A and LY-294002) also extend lifespan in N2 worms in a CR-like manner (Figure 1). An increase in healthspan was also observed [9].

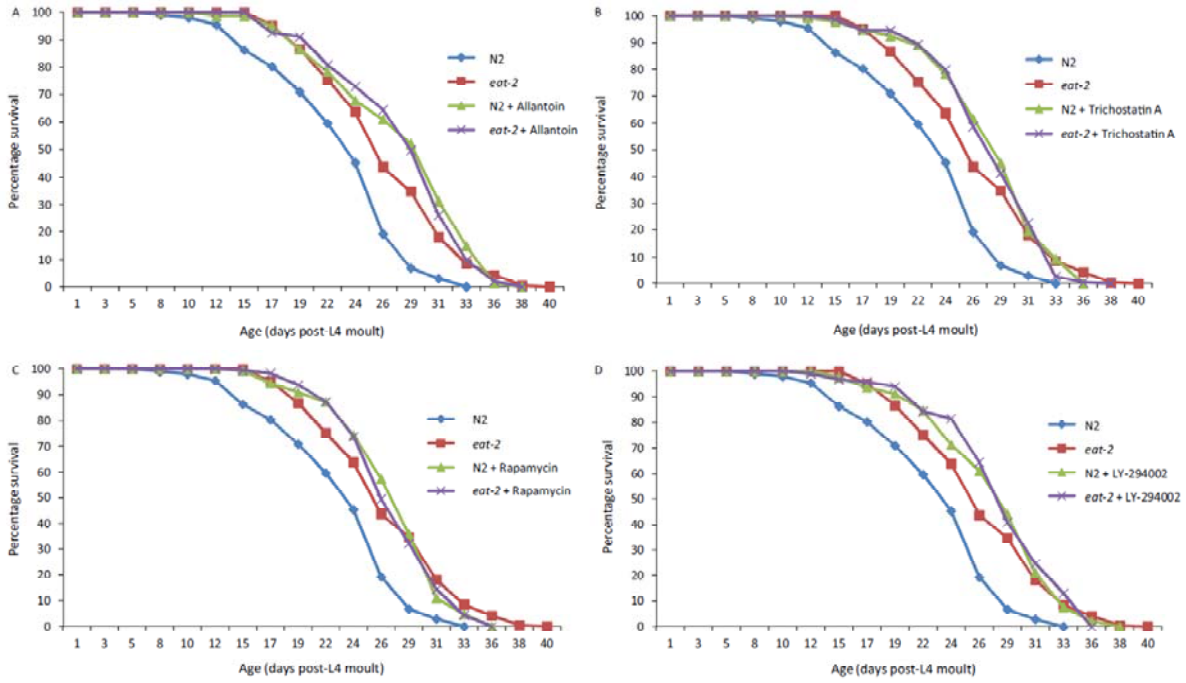

Figure 1: Preliminary work, published in [9], in C. elegans *showing that allantoin (A), trichostatin A (B), rapamycin (C) or LY-294002 (D) extend lifespan (statistically significant according to Log-rank test) in N2 controls worms but not in* eat-2 *mutants, a genetic model of CR.*

These results demonstrate that our network pharmacology approach can reveal new CR mimetics. They also show that we can perform lifespan and healthspan experiments in worms.

Worm model of adult-onset neuronal ceroid lipofuscinosis (ANCL): Our local collaborator in Liverpool (Prof Alan Morgan) recently developed a *C. elegans dnj-14* null mutant model of the human neurodegenerative disease ANCL. This model exhibits a shortened lifespan and age-dependent sensory neuron degeneration and provides a new platform for neuroprotective drug screening [15]. As proof-of-concept, Prof Morgan showed that resveratrol could ameliorate *dnj-14* mutant phenotypes, including lifespan [15]. Therefore, this will be one of the models we will employ for assaying neuroprotective effects.

Taken together, our preliminary results demonstrate that our computational predictions can help guide ageing studies and that we can perform the proposed experiments in worms. *C. elegans* is ideal for this project given its short lifespan (studies in rodents would not be feasible or cost-effective for so many candidate compounds) and, in fact, has been widely used to study ageing, CR and neurodegeneration [9, 11, 17, 18]. Indeed, findings in worms underlie major current efforts to translate basic ageing biology findings to the clinic [8, 11]. Besides, simple model organisms, like worms, are increasingly recognized as major paradigms to study conserved mechanisms involved in neurodegeneration [19], and *C. elegans* has been, in fact, widely used to study neurodegenerative conditions [18] and discover new therapeutic compounds for such conditions [17]. Although many compounds studied in worms are only effective in single neurodegenerative models, some compounds, like resveratrol which has been argued as capable of retarding ageing, can be neuroprotective in several worm models and even in mammalian systems [15, 16]; these results provide proof-of-concept that it is possible to identify compounds with general neuroprotective effects.

3) Aims and purposes of proposed investigation

In this project, we aim to study the 11 new candidate CR mimetics in two worm models of neurodegenerative diseases: a *dnj-14* null mutant model of adult-onset neuronal ceroid lipofuscinosis (ANCL) and a worm model of frontotemporal dementia expressing an human Tau mutant, parkinsonism-17. We expect to identify compounds with potential neuroprotective effects that will be promising for follow-up in mammalian models. Although some of our 11 compounds are known not to be suitable for human use, we will still study them to identify novel drug classes suitable as CR mimetics that may be important for further studies.

This project builds upon the PI's lab expertise in ageing (http://pcwww.liv.ac.uk/~aging/). I will collaborate with Profs Andrew Cossins and Alan Morgan in Liverpool who have experience working with *C. elegans*. Prof Morgan will provide the *C. elegans* models to study human age-related neurodegenerative disorders.

4) Plan of investigation and methodology

All 11 candidate CR mimetic compounds identified thus far will be assayed. We will first assay the compounds for their ability to increase the short lifespan of each of the mutants. For drugs extending the lifespan of *dnj-14* mutants, we will then also test if they are able to rescue the sensory defect observed in these animals by performing a food race assay. Likewise, we will perform thrashing assays to determine if drugs improve the uncoordinated phenotype of Tau transgenic worms.

Methodologies —

We will employ two worm mutant models: the aforementioned *dnj-14* null mutant model and an established worm model of frontotemporal dementia expressing an human Tau mutant, parkinsonism-17 (FTDP-17) [20]. This tauopathy model, already being studied in Liverpool, also exhibits a short lifespan as well as

severe motility defects which are highly penetrant and easily observable and therefore suitable for assessing drug effects [21].

We will first assay the 11 candidate compounds for their ability to increase the short lifespan of each of the mutants. Worms (N2 Bristol) will be cultured under standard conditions [22]. N2 is the most widely used strain and we will use the CGC male stock, as recommended [23]; indeed, our controls do not have reduced lifespan (Figure 1), which has been a problem in other studies [23]. (Assays in other worm strains could be conducted if deemed cost-effective or in future projects.) Worms will be fed UV-killed OP50 to minimize any biases from drug effects on bacteria (this protocol has already been optimized in our lab). The drug dosages from the Connectivity Map which most significantly overlap with CR signatures will be used initially, though dose-dependent experiments will be performed to determine optimal concentrations since often higher concentrations are necessary in worms when compared to cell cultures; it is also possible that there are dose-dependent neuroprotective effects. Experiments will be performed in triplicate, by standard mortality curves, using 40-60 worms per well [13, 17]. Standard lifespan statistics will be performed [13].

For compounds extending the lifespan of *dnj-14* mutants, we will then also test if they are able to rescue the sensory defect observed in these animals by performing chemotaxis assays, as we previously described [15]. Likewise, we will perform thrashing assays to determine if compounds improve the uncoordinated phenotype of Tau transgenic worms. Standard statistical tests [15] will be performed.

We have the equipment, strains and staff in place to carry out these experiments. Therefore, we can start this project right away should it be funded. It will take 24 months to be completed (see timeline below).

Timeline and work plan: Testing the 11 compounds for lifespan effects in the ANCL worm model (*dnj-14* mutants) is expected to be completed in the first 6 months of the project, followed by testing the 11 compounds for lifespan effects in the frontotemporal dementia worm model (months 6-12). Then for compounds extending the lifespan in the ANCL worm model, we will test if they are able to rescue the sensory defect observed in these animals (months 12-18). For compounds extending the lifespan in the frontotemporal dementia worm model, we will perform thrashing assays to determine if compounds improve the uncoordinated phenotype of the transgenic worms (months 18-24).

Project work plan

Milestone # and Tasks		**Months**				
		0	**6**	**12**	**18**	**24**
1	Test compounds for lifespan effects in ANCL model	■				
2	Test compounds for lifespan effects in dementia model		■			
3	Assay life-extending compounds in ANCL model for health			■		
4	Assay life-extending compounds in dementia model for health				■	

Milestones:

1) 6 months: identify which compounds extend lifespan and by which extent in a worm model of adult-onset neuronal ceroid lipofuscinosis.

2) 12 months: identify which compounds extend lifespan and by which extent in a worm model of frontotemporal dementia.

3) 18 months: for drugs extending lifespan in adult-onset neuronal ceroid lipofuscinosis, identify which also ameliorate health.

4) 24 months: for drugs extending lifespan in frontotemporal dementia, identify which also ameliorate health.

Future plans and outlook

There is considerable current interest in repurposing drugs for the treatment of neurodegenerative conditions. We expect to identify new drugs and drug classes with potential for the treatment of neurodegenerative diseases that we can then take on to further and longer studies in mammalian models. This may require substantial additional funding, however. In this context, the proposed pilot project will provide essential pilot data for a larger grant application to translational funding streams from the research councils (e.g. MRC with its Developmental Pathway Funding scheme) or Wellcome Trust (Seeding Drug Discovery scheme) or to charities like Parkinson's UK and Alzheimer's Society. We will also engage companies for potential exploitation and partnerships.

5) Budget

Consumables (£9,000 per year): Funds are requested to purchase the drugs to be tested (many of which are quite expensive) and also to purchase standard worm culture reagents, strains, materials and consumables for the duration of this project.

6) References

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