A triple drug combination targeting components of the nutrient-sensing network maximizes longevity

Jorge Iván Castillo-Quan,a,b,c,d,1 Luke S. Tain,a,b,1 Kerri J. Kinghorn,a,b, Li Li,a,b, Sebastian Grönke,c, Yvonne Hinze,d T. Keith Blackwell,b,c, Ivana Bjedov,a,d, and Linda Partridgea,b,d

aInstitute of Healthy Ageing, Department of Genetics, Evolution and Environment, University College London, WC1E 6BT London, United Kingdom; bSection on Islet Cell & Regenerative Biology, Joslin Diabetes Center, Boston, MA 02215; cDepartment of Genetics, Harvard Medical School, Boston, MA 02115; dDepartment of Biological Mechanisms of Ageing, Max Planck Institute for Biology of Ageing, D-50931 Cologne, Germany; eDepartment of Molecular Neuroscience, Institute of Neurology, WC1N 3BG London, United Kingdom; and fDepartment of Cancer Biology, Cancer Institute, University College London, WC1E 6DD London, United Kingdom

Edited by Joseph S. Takahashi, The University of Texas Southwestern Medical Center, Dallas, TX, and approved September 16, 2019 (received for review August 1, 2019)

Increasing life expectancy is causing the prevalence of age-related diseases to rise, and there is an urgent need for new strategies to improve health at older ages. Reduced activity of insulin/insulin-like growth factor signaling (IIS) and mechanistic target of rapamycin (mTOR) nutrient-sensing signaling network can extend lifespan and improve health during aging in diverse organisms. However, the extensive feedback in this network and adverse side effects of inhibition imply that simultaneous targeting of specific effectors in the network may most effectively combat the effects of aging. We show that the mitogen-activated protein kinase kinase (MEK) inhibitor trametinib, the mTOR complex 1 (mTORC1) inhibitor rapamycin, and the glycogen synthase kinase-3 (GSK-3) inhibitor lithium act additively to increase longevity in Drosophila. Remarkably, the triple drug combination increased lifespan by 48%. Furthermore, the combination of lithium with rapamycin cancelled the latter’s effects on lipid metabolism. In conclusion, a polypharmacology approach of combining established, prolongevity drug inhibitors of specific nodes may be the most effective way to target the nutrient-sensing network to improve late-life health.

Aging | polypharmacology | trametinib | rapamycin | lithium

Aging is a complex process of progressive cell, tissue, and systemic dysfunction that is involved in the etiology of age-related diseases (1). Genetic, dietary, and pharmacological interventions can ameliorate the effects of aging in laboratory animals and may lead to therapies against age-related diseases in humans (2–4).

In organisms ranging from invertebrates to mammals, reducing the activity of the nutrient-sensing mechanistic target of rapamycin (mTOR) and insulin/insulin-like growth factor signaling (IIS) network can promote longevity and health during aging (2, 3). Lowering network activity can also protect against the pathology associated with genetic models of age-related diseases (1, 2). The network contains many drug targets, including mTOR, mitogen-activated protein kinase kinase (MEK), and glycogen synthase kinase-3 (GSK-3) (Fig. 1A). Down-regulation of mTOR activity by rapamycin, GSK-3 by lithium, or MEK by trametinib can each individually extend lifespan in laboratory organisms (5–11), and brief inhibition of mTOR has recently been shown to increase the response of elderly people to immunization against influenza (12). In addition, both mTOR and MEK inhibitors have been shown to reduce senescent phenotypes in human cells (13), while increasing concentrations of lithium levels in drinking water correlate with reduced all-cause mortality in a Japanese population (10). An advantage of pharmacological interventions is that the timing and dose of drug administration are relatively simple to optimize, and drugs can be easily combined (4, 14–16). Combination drug treatments also have the potential to counter resistance from feedback and to reduce each other’s side effects (17). Rapamycin, trametinib, and lithium each target different kinases and transcription factors to extend lifespan (5, 8, 11), and therefore their effector mechanisms are at least partially different from each other. Simultaneous inhibition of multiple targets within the nutrient-sensing network may hence be needed to optimize effector outputs and health benefits. Here, we measure the effects of combination treatments of rapamycin, lithium, and trametinib on lifespan and other traits, using Drosophila as a model organism.

Results and Discussion

Rapamycin treatment, from Caenorhabditis elegans to humans, is associated with altered metabolism, including hypertriglyceridemia and obesity (5, 18). Alone, a lifespan-extending dose of lithium (11) did not alter triglyceride levels, but simultaneous treatment with both lithium and rapamycin reversed the dyslipidemia caused by rapamycin (Fig. 1B). To confirm that this change in lipid levels was physiologically relevant, we pretreated (14 d) flies with lithium, rapamycin, or a combination, and assessed their survival under starvation. Lithium did not alter survival under starvation conditions, while rapamycin increased it (Fig. 1C). Consistent with their effects on lipid levels, combining lithium and rapamycin treatment resulted in control levels of starvation resistance (Fig. 1C). Lithium can therefore reverse metabolic storage alterations associated with mTOR inhibition.

Lithium inhibits GSK-3 activity to extend lifespan (11), implying that activation of GSK3 is likely, if anything, to shorten lifespan. Inhibition of IIS in the canonical PI3K pathway can extend lifespan and health span, but reduces inhibitory phosphorylation of GSK3 by Akt (Fig. 1A), and hence activates GSK3 (4), a potentially deleterious side effect of lowered IIS (19). We therefore tested whether lithium could have additive effects in combination with genetic inhibition of IIS upstream of Akt. Lithium was able to further extend the lifespan of flies lacking the insulin-like peptides 2, 3, and 5 (dilp2-3,5) (Fig. 1D) (20). In contrast, rapamycin or trametinib, neither of which inhibit GSK3, were not able to extend the lifespan of dilp2-3,5 flies (Fig. 1E and

Author contributions: J.I.C.-Q. and L.P. designed research; J.I.C.-Q., K.J.K., L.L., S.G., Y.H., and I.B. performed research; J.I.C.-Q. and L.S.T. analyzed data; J.I.C.-Q., L.S.T., and L.P. wrote the paper; T.K.B. provided input in manuscript writing; J.I.C.-Q., L.S.T., and L.P. interpreted data; and T.K.B. and L.P. supervised experiments. The authors declare no competing interest.

This open access article is distributed under Creative Commons Attribution License 4.0 (CC BY).

J.I.C.-Q. and L.S.T. contributed equally to this work.

1Present address: Department of Neurosurgery, School of Medicine, Stanford University, Palo Alto, CA 94304.

2To whom correspondence may be addressed. Email: l.partridge@ucl.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1913212116/-/DCSupplemental.

First published September 30, 2019.
Lithium reversed this and explained the greater longevity observed with the triple drug combination.

Given the complex nature of the aging process, it is unlikely that the most effective preventative antiaging therapy could be achieved by a single compound with a single target. We have shown that simultaneous inhibition by 3 components of different nodes in the nutrient-sensing network using a combination of drugs already approved for human use is a viable strategy to maximize animal longevity and to reduce a side effect. Rapamycin treatment results in insulin resistance and dyslipidemia in patients and mice (4, 18, 22), and this disturbance manifests as hypertriglyceridemia in Drosophila (5). Lithium reversed this and the starvation resistance associated with rapamycin treatment. Taken together, our results highlight a potential therapeutic avenue to promote longevity, coadministering compounds that act on different nodes of the nutrient-sensing network, to maximize their beneficial effects while minimizing negative side effects.

Methods

Fly Stocks, Husbandry, and Lifespan Analysis. For all experiments, a wild-type white D. melanogaster stock, or, when noted, dilp2-3,5 mutant flies (wMelPop background), were used, and raised as previously described (20). Lithium (Sigma) in ddH2O, trametinib (LC laboratories) in dimethyl sulfoxide, and rapamycin (LC laboratories) in 100% ethanol were added to sugar–yeast–agar (SYA) medium to a final concentration of 1 mM, 15.6 μM, and 50 μM, respectively (5, 8, 11). Equivalent volumes and concentrations of vehicle were added to SYA medium for control treatments. Drug treatments were started 2 d posteclosion. Female flies (n = 130 to 200, 15 to 20 per vial) were sorted onto SYA medium that was replaced every 2 to 3 d throughout life.

Fig. 1. Lithium blocks negative side effects of mTORC1 and IIS inhibition. (A) A simplified diagram of the Drosophila nutrient-sensing network showing the target kinases of rapamycin, trametinib, and lithium. Lithium reversed (B) hyperlipidemia (n = 6 replicas of 5 flies per condition, 1-way ANOVA and (C) starvation resistance induced by rapamycin (50 μM) (n = 75). (D) Lithium treatment significantly extended lifespan of both wMelPop and dilp2-3,5 mutant flies. Neither (E) rapamycin (P = 0.58) nor (F) trametinib (P = 0.14) further extended lifespan of dilp2-3,5 mutant flies (log-rank test, n = 150). Cox Proportional Hazard analysis showed a significant genotype by treatment interaction for rapamycin (P = 0.002) and trametinib (P = 0.0018). Error bars represent SEM. ***P < 0.001 (1-way ANOVA or log-rank test).

Fig. 2. A triple drug combination maximizes longevity. (A) Representative survival curve and associated pairwise log-rank tests. (B) Replicated median/maximum lifespans plotted for all single (n = 4), double (n = 3), and triple (n = 2) combinations of rapamycin, trametinib, and lithium treatments. Each lifespan contained 130 to 200 flies per treatment. Numbers in parentheses show (total number of flies/number of censors). (C) Proboscis extension feeding behavior assay (1 and 15 d of treatment; Top and Middle) and quantification of ingested nonabsorbable (Bottom) blue dye (n = 8 replicas of 4 to 5 flies 15 d old, 1-way ANOVA with Dunnett’s test). (D) Mass spectrometry of systemic trametinib (Top) or rapamycin (Bottom) levels when other drugs were coadministered (n = 5, 1-way ANOVA). (E) Fecundity of treated (15 d) flies within a 24-h period (n = 8 replicas of 4 to 5 flies). Error bars show Tukey whiskers, and outlying data points are shown as dots. *P < 0.05, **P < 0.01, ***P < 0.001 (Kruskal–Wallis test and Dunn’s pairwise tests).
Lifespan raw data are provided as Dataset S1. Starvation assay was performed as previously described (11).

Food Intake, Fecundity, and Triglyceride Measurements. Feeding behavior (proboscis extension at 1 and 15 d of treatment) and food intake (quantified by dye-calibrated feeding) (4 to 5 flies per replicate, n = 8 to 10) were measured as previously described (23). Fecundity was quantified as number of eggs laid within 24 h (15 d), and triglyceride measurements (5 flies per replicate, n = 8) were performed as previously described (5, 11).

Mass Spectrometry. Flies (n = 5, 15 flies) were treated with drugs (15 d), their digestive system was allowed to void (1 h), they were snap frozen, drugs were extracted as previously described (5), and they were resuspended in 100 μL of acetonitrile/isopropanol 70:30 for measurement with an Acquity UPLC i-class System/Xevo TQ-S (Waters) with MassLynx and absolute quantification.

ACKNOWLEDGMENTS. We are grateful to Prof. David Gems and Drs. Helena Cochréme, Natalie Moroz, and Filipe Cabrero for advice and comments, and to Rachel Beltzhoover for proofreading. We thank Drs. Fiona Kerr, Anna Tillmann, and Giovanna Vinti for technical advice and assistance. We acknowledge funding from University College London London Scholarships (J.I.C.-Q), American Federation for Aging Research/Gilen Foundation for Medical Research Postdoctoral Fellowship (Grant PD18019 to J.I.C.-Q), Max Planck Society (J.I.C.-Q, L.S.T., S.G., Y.H., and L.P.), and National Institutes of Health (Grants AG045215 and GM122610 to T.K.B.). This project has received funding from the European Research Council under the European Union’s Horizon 2020 research and innovation program (Grant Agreement 741989). European Research Council Starting Grant (Grant 311331 to I.B.), Research Into Ageing (J.B. and L.P.), Parkinson’s UK (L.L. and L.P.), Wellcome Trust Clinical Career Development Fellowship (Grant 214589/Z/18/Z to K.J.K.), Wellcome Trust Strategic Award (WT098565/Z/12/Z to L.P.), and Academy of Medical Sciences (K.J.K.).

1. C. López-Otín, M. A. Blasco, L. Partridge, M. Serrano, G. Kroemer, The hallmarks of aging. Cell 153, 1194–1217 (2013).
2. J. Campisi et al., From discoveries in ageing research to therapeutics for healthy ageing. Nature 571, 183–192 (2019).
3. C. J. Kenyon, The genetics of ageing. Nature 464, 504–512 (2010).
4. J. I. Castillo-Quan, K. J. Kinghorn, L. Bjedov, Genetics and pharmacology of longevity: The road to therapeutics for healthy aging. Adv. Genet. 90, 1–101 (2015).
5. L. Bjedov et al., Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster. Cell Metab. 11, 35–46 (2010).
6. D. E. Harrison et al., Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395 (2009).
7. S. Robida-Stubbbs et al., TOR signaling and rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. Cell Metab. 15, 713–724 (2012).
8. C. Slack et al., The Ras-Erk-ETS-signaling pathway is a drug target for longevity. Cell 162, 72–83 (2015).
9. G. McColl et al., Pharmacogenetic analysis of lithium-induced delayed aging in Cae-norhabditis elegans. J. Biol. Chem. 283, 350–357 (2008).
10. K. Zane et al., Low-dose lithium uptake promotes longevity in humans and metazoans. Eur. J. Nutr. 50, 387–389 (2011).
11. J. I. Castillo-Quan et al., Lithium promotes longevity through GSK3/NRF2-dependent hormesis. Cell Rep. 15, 638–650 (2016).
12. J. B. Mannick et al., mTOR inhibition improves immune function in the elderly. Sci. Transl. Med. 6, 268ra179 (2014).
13. Z. N. Demidenko, M. Shituman, M. V. Blagosklonny, Pharmacologic inhibition of MEK and PI-3K converges on the mTOR/S6 pathway to decelerate cellular senescence. Cell Cycle 8, 1896–1900 (2009).
14. T. D. Admasu et al., Drug synergy slows aging and improves healthspan through IGF and SREBP lipid signaling. Dev. Cell 47, 67–79.e5 (2018).
15. P. Dakik et al., Pairwise combinations of chemical compounds that delay yeast chronological aging through different signaling pathways display synergistic effects on the extent of aging delay. Oncotarget 10, 313–338 (2019).
16. K. Evanson, J. J. Collins, C. Huang, S. Hughes, K. Kornfeld, Valproic acid extends Cae-norhabditis elegans lifespan. Aging Cell 7, 305–317 (2008).
17. B. D. Levine, R. L. Cagan, Drosophila lung cancer models identify trametinib plus statin as candidate therapeutic. Cell Rep. 14, 1477–1487 (2016).
18. V. P. Houde et al., Chronic rapamycin treatment causes glucose intolerance and hyperlipidemia by upregulating hepatic gluconeogenesis and impairing lipid deposition in adipose tissue. Diabetes 59, 1338–1348 (2010).
19. K. Tanabe et al., Genetic deficiency of glycolgen synthase kinase-3beta corrects diabetes in mouse models of insulin resistance. PLoS Biol. 6, e37 (2008).
20. S. Grönke, D. F. Clarke, S. Broughton, T. D. Andrews, L. Partridge, Molecular evolution and functional characterization of Drosophila insulin-like peptides. PLoS Genet. 6, e1000857 (2010).
21. M. Jafari, Drosophila melanogaster as a model system for the evaluation of anti-aging compounds. Fly (Austin) 4, 253–257 (2010).
22. D. W. Lamming et al., Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. Science 335, 1638–1643 (2012).
23. R. Wong, M. D. W. Piper, B. Wertheim, L. Partridge, Quantification of food intake in Drosophila. PLoS One 4, e6063 (2009).