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In Focus

NIA Interventions Testing Program: Investigating Putative Aging Intervention Agents in a Genetically Heterogeneous Mouse Model

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The Interventions Testing Program (ITP) was established by the National Institute on Aging (NIA) to investigate the potential of dietary interventions to promote healthy aging (https://www.nia.nih.gov/research/dab/interventions-testing-program-itp). The ITP uses a four-way cross genetically heterogeneous mouse model (UM-HET3) to reduce the impact of strain-specific characteristics on outcomes (Nadon et al., 2008). Lifespan tests are done in parallel, using the same protocol, at three independent sites to increase robustness of the findings. Population sizes are large enough that the protocol will detect a 10% change in mean lifespan, in either sex, with 80% power, pooling data from as few as two sites. Standard operating procedures were designed to maintain as much consistency as possible among the three sites, including caging, bedding, food, and light/dark cycles; a more in-depth discussion of the SOP has been published (Nadon et al., 2015). Interventions for testing are proposed by the research community through an annual call-for-proposals, and proposed compounds have ranged from drugs and dietary supplements to micronutrients and metabolic intermediates.

Before the ITP embarks on testing a compound, pilot studies are done to maximize the chances of a successful test. Goals of the pilot studies include demonstrating that the compound is stable in food and that it is uniformly mixed in the food, determining blood levels after short-term treatment (bioavailability), showing evidence of an effect from the short-term treatment (bioactivity), and in some cases, testing for toxicity. The testing of rapamycin is a good case-in-point for analyzing stability of the compound in the food. Pilot analysis showed that about 85% of the rapamycin was degraded by the food preparation process, leading to the use of microencapsulation to deliver stable doses of the compound in food (Harrison et al., 2009).

The list of all compounds tested by the ITP and in progress is on the ITP website at https://www.nia.nih.gov/research/dab/interventions-testing-program-itp/compounds-testing. To date, six compounds have shown significant extension of lifespan:

- Aspirin – males only (Strong et al., 2008);
- Rapamycin – males and females (females > males) (Harrison et al., 2009; Miller et al., 2011; Miller et al., 2014);
- 17αEstradiol – males only (Harrison et al., 2014);
- Acarbose – males and females (males> > females) (Harrison et al., 2014);
- Nordihydroguaiaretic acid (NDGA) – males only (Strong et al., 2008; Harrison et al., 2014);
- Protandim® - males only (Strong et al., 2016).

The positive findings illustrate some important aspects for aging interventions research. The effective interventions appear to include several disparate mechanisms, demonstrating that many cellular pathways might be exploited to influence lifespan and aging. Rapamycin modulates the nutrient-sensing pathways via its interaction with mTOR (Harrison et al., 2009). Acarbose was anticipated to work as a caloric restriction mimetic due to its ability to reduce the rate of absorption of carbohydrates, but its mechanism of action appears more complex, since caloric restriction results in significant lifespan extension in both male and female UM-HET3 mice (Flurkey et al., 2010), while the effects of acarbose were much larger in males (Harrison et al., 2014). Aspirin is known for its anti-inflammatory and antioxidant activities, NDGA also has anti-inflammatory and antioxidant activities, 17αEstradiol has neuro-protective properties independent of binding to the estrogen receptor, and Protandim® activates Nrf2 transcriptional regulator (Strong et al., 2008, 2016). This diverse group of interventions demonstrates the complex nature of the biology of aging.

Another major surprise is the extent of sex differences in response to the interventions. Four of the six positive interventions only worked in one sex, and the two that had an effect in both sexes showed sex-specific differences in the extent of the effect. Blood levels of a compound

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sometimes differed between males and females, but that did not always explain the sex difference in lifespan extension. For rapamycin, achieving approximately equivalent blood levels in males and females by treating with different doses did result in similar increases in lifespan (Miller et al., 2014). But for NDGA, even at doses giving similar blood levels in males and females, females still did not respond (Harrison et al., 2014). The ITP's findings illustrate how important it is to examine the effects of interventions in both sexes and suggest that further studies on the mechanism of these sex effects may yield important insights into the underlying biology, and guidance for eventually clinical studies.

Lifespan, while a valuable measurement in rodent model studies, does not entirely capture the impact of aging interventions. Aging is a complex process, with many physiological systems affected, and not all at the same time or rate. Measurements of a wide range of endpoints relevant to health and maintenance of function will help to clarify the relationship of aging to function and disease, and may also document drug effects pertinent to human health maintenance. The ITP is developing a panel of measurements to assess health and function that will be used across cohorts to add to the information the lifespan studies provide.

Studies in the mouse have contributed significantly to our understanding of aging and interventions to promote healthy aging. Testing both sexes and avoiding the use of a single inbred strain are key features that will enhance translation of the findings. A power analysis is essential to get the most value from experiments, and standardized protocols allow for cross-experimental comparisons. Multi-site testing protocols also add value to the design because some site-to-site variation is unavoidable even with every effort made to minimize differences between sites. For example, the ITP has consistently found that control male mice at one site weigh less and live longer than the control males at the other two sites, even though each site uses the same food preparations and standardized husbandry (Strong et al., 2012). Positive findings replicated in different labs are inherently stronger than a finding from one lab, while disparate findings convey a valuable caution and emphasize the need for replications in other laboratories, other mouse stocks, and other drug doses.

In summary, the ITP program, now in its 13th year, has become a major contributor to the biogerontology research community, providing new insights into the biology of aging, new tools for probing the relationships among cells, aging, and disease, and new ideas about how best to translate studies in basic biogerontology into human investigation, and, eventually, into the clinic.

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References

Flurkey, K., Astle, C.M., Harrison, D.E., 2010. Life extension by diet restriction and *N*-acetyl-L-cysteine in genetically heterogeneous mice. J. Gerontol. A Biol. Sci. Med. Sci. 65, 1275–1284.

Harrison, D.E., Strong, R., Sharp, Z.D., Nelson, J.F., Astle, C.M., Flurkey, K., Nadon, N.L., Wilkinson, J.E., Frenkel, K., Carter, C.S., Pahor, M., Javors, M.A., Fernandez, E., Miller, R.A., 2009. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460, 392–395.

Harrison, D.E., Strong, R., Allison, D.B., Ames, B.N., Astle, C.M., Atamna, H., Fernandez, E., Flurkey, K., Javors, M.A., Nadon, N.L., Nelson, J.F., Simpkins, J.W., Smith, D., Wilkinson, J.E., Miller, R.A., 2014. Acarbose, 17-α-estradiol, and nordihydroguaiaretic acid extend mouse lifespan preferentially in males. Aging Cell 13, 273–282.

Miller, R.A., Harrison, D., Astle, C.M., Baur, J.A., deCabo, R., Fernandez, E., Flurkey, K., Javors, M.A., Nelson, J.F., Pletcher, S., Sharp, Z.D., Sinclair, D., Starnes, J.W., Wilkinson, J.E., Nadon, N.L., Strong, R., 2011. Rapamycin, but not resveratrol or simvastatin, extends lifespan of genetically heterogeneous mice. J. Gerontology, Biol. Sci. 66A, 191–201.

Miller, R.A., Harrison, D.E., Astle, C.M., Fernandez, E., Flurkey, K., Han, M., Javors, M.A., Li, X., Nadon, N.L., Nelson, J.F., Pletcher, S., Salmon, A.B., Sharp, Z.D., Van Roekel, S., Winkleman, L., Strong, R., 2014. Rapamycin-mediated lifespan increase in mice is dose and sex-dependent and metabolically distinct from dietary restriction. Aging Cell 13, 468–477.

Nadon, N.L., Strong, R., Miller, R.A., Nelson, J., Javors, M., Sharp, Z.D., Peralba, J.M., Harrison, D.E., 2008. Design of aging intervention studies: the NIA interventions testing program. Age 30, 187–199.

Nadon, N.L., Miller, R.A., Strong, R., Harrison, D.E., 2015. NIA interventions testing program: a collaborative approach for investigating interventions to promote healthy aging. In: Kaeberlein, M.R., Martin, G.M. (Eds.), Handbook for the Biology of Aging, eighth ed., pp. 287–303.

Strong, R., Miller, R.A., Astle, C.M., Floyd, R.A., Flurkey, K., Hensley, K.L., Javors, M.A., Leeuwenburgh, C., Nelson, J.F., Ongini, E., Nadon, N.L., Warner, H.R., Harrison, D.E., 2008. Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice. Aging Cell 7, 641–650.

Strong, R., Miller, R.A., Astle, C.M., Baur, J.A., de Cabo, R., Fernandez, E., Guo, W., Javors, M., Kirkland, J.L., Nelson, J.F., Sinclair, D., Teter, B., Williams, D., Zaveri, N., Nadon, N.L., Harrison, D.E., 2012. Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid, and medium chain triglyceride oil on lifespan of genetically heterogeneous mice. J. Gerontol. Biol. Sci. 68, 6–16.

Strong, R., Miller, R.A., Antebi, A., Astle, C.M., Bogue, M., Denzel, M.S., Fernandez, E., Flurkey, K., Hamilton, K.L., Lamming, D.W., Javors, M.A., de Magalhães, J.P., McCord, J.M., Miller, B.F., Müller, M., Nelson, J.F., Ndukum, J., Rainger, G.E., Richardson, A., Sabatini, D.M., Salmon, A.B., Simpkins, J.W., Steegenga, W.T., Nadon, N.L., Harrison, D.E., 2016. Longer lifespan in male mice treated with a weakly-estrogenic agonist, an antioxidant, an α-glucosidase inhibitor or a Nrf2-inducer. Aging Cell http://dx.doi.org/10.1111/acel.12496.