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Post-dauer Life Span of *Caenorhabditis Elegans* Dauer Larvae Can be Modified by X-irradiation

Akira ONODERA[1], Sumino YANASE[1,2], Takamasa ISHII[1], Kayo YASUDA[3], Masaki MIYAZAWA[1], Philip S. HARTMAN[4] and Naoaki ISHII[1*]

Aging/*C. elegans*/Dauer larvae/Radiation/Beneficial effect.

The time spent as a dauer larva does not affect adult life span in *Caenorhabditis elegans*, as if aging is suspended in this quiescent developmental stage. We now report that modest doses X-irradiation of dauer larvae increased their post-dauer longevity. Post-irradiation incubation of young dauer larvae did not modify this beneficial effect of radiation. Conversely, holding dauer larvae prior to irradiation rendered them refractory to this X-radiation-induced response. We present a model to explain these results. These experiments demonstrate that dauer larvae provide an excellent opportunity to study mechanisms by which X irradiation can extend life span.

INTRODUCTION

Under adverse environmental conditions such as a high population density or starvation, *Caenorhabditis elegans* development can adopt a quiescent stage called the dauer larva.[1)] Dauer larvae consume comparatively little energy, and their metabolism differs markedly from all other stages.[2)] They can persist for months only to resume normal development when presented with food. In 1976, Klass and Hirsh demonstrated that the time spent as a dauer larva did not affect the post-dauer life span, giving the impression that dauer larvae suspend the process of aging.[3)] However, Houthoofd and his colleagues have demonstrated that dauer larvae do "age" in that their metabolism gradually declines, and they slowly accumulate senescent-associated lipofuscins.[4)] Dauers eventually "die" in the sense that they lose their ability to recover to adulthood, even when presented with adequate food. One of our collaborators, Hartman noted that the UV radiation sensitivity of dauer larvae was roughly equivalent to other larval stages.[5)] In addition, young and old dauer larvae were equally sensitive to UV radiation. Finally, survival did not increase when dauer larvae were held in buffer after irradiation. However, these experiments only measured the effects of irradiation on development and did not address its effects on life span.

Hormesis is the ability of low-level exposure to a normally harmful agent to cause a positive effect, usually that of life-span enhancement. This is likely the result of up-regulation of antioxidant enzymes that confer resistance to subsequent, more profound insult such as oxidative stress. A variety of stressors can promote hormesis in *C. elegans*, including in dauer larvae.[6–13)] For example, oxidative stress pretreatment increases the X-irradiation resistance of *C. elegans*.[9)] Interestingly, dauer larvae have been reported to express increases in total SOD activity and the mitochondrially located MnSOD gene *sod-3*.[14,15)] This suggests that this particular developmental variant might be especially useful for the study of beneficial effects such as hormesis.

We now report that X-irradiation can significantly extend the life span of dauer larvae. Moreover, the effect can be modulated by varying the incubation periods between X-ray exposure and recovery to adulthood.

MATERIALS AND METHODS

General methods

C. elegans N2, Bristol (wild type) was obtained from the Caenorhabditis Genetics Center (Minnesota, U.S.A). Animals were cultivated on nematode growth medium (NGM) agar plates seeded with *Escherichia coli* OP50 as previously described by Brenner.[16)] Dauer larvae were isolated by the method of Wang and Kim and stored in S-buffer [100 mM

*Corresponding author: Phone: +81-463-93-1121 ext. 2651,
Fax: +81-463-94-8884,
E-mail: nishii@is.icc.u-tokai.ac.jp
[1]Department of Molecular Life Science, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan; [2]Department of Health Science, Daito Bunka University School of Sports & Health Science, Higashi-Matsuyama, Saitama 355-8501, Japan; [3]Teaching and Research Support Center, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan; [4]Biology Department, Texas Christian University, Fort Worth, TX 76129, USA.
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NaCl, 50 mM potassium phosphate (pH 6.0)] at 25°C.[17] We then systemically explored the effects of holding dauer larvae for various times before irradiation (pre-irradiation) as well as after irradiation and before presentation of bacteria (post-irradiation), which triggers exit from the dauer stage and resumption of normal development. This approach is schematically represented in Fig. 1, as "X days" represents the time between dauer-larvae isolation and irradiation while

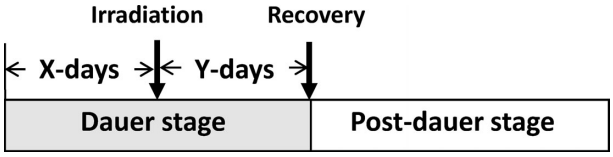

Fig. 1. Scheme depicting the timing of irradiation and recovery, with X and Y days referring to various times either pre- or post-irradiation but before recovery, respectively.

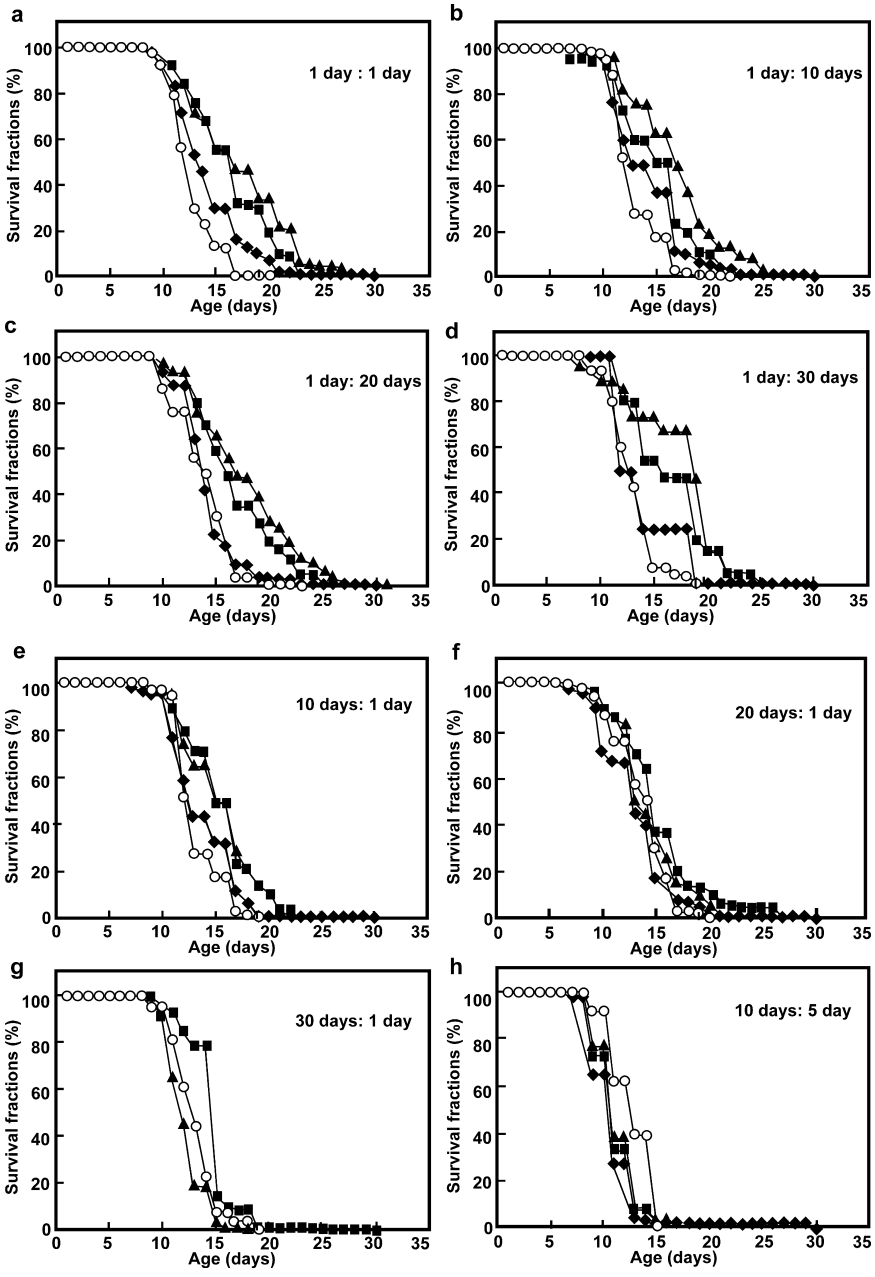

Fig. 2. Life spans of post-dauer larvae exposed to 0 (open circles), 100 (closed triangles), 300 (closed squares) or 500 Gy (closed diamonds) of X-rays. The age refers to the time of exposure to *E. coli*, which triggers exit from the dauer stage. In each panel the first number indicates the number of days between dauer isolation and irradiation (pre-irradiation time : X days) while the second number indicates the number of days between irradiation and exposure to *E. coli* (post-irradiation time : Y days).

"Y days" represents the time after irradiation before food presentation.

X-irradiation

Animals were irradiated on NGM agar plates. Irradiation with X-rays was performed with a generator (50 kVp, 5 mA, 0.05 mm Aluminum filter, Softx Co. Ltd., Japan, OMC-605 R) at a dose rate of 2.0 Gy per min. The dose was measured with a Fricke chemical dosimeter.

Life spans of post-dauer larvae

Each value indicates the mean of three separate experiments. About 100 dauer larvae were used for each sample. The data of life-span values were subjected to one-way analysis of variance (ANOVA) by age of worms or dose of X-rays, and statistical comparisons of mean life-span values among different age or dose were determined by Tukey's multiple-range test.

RESULTS

A strong beneficial effect was observed when dauer larvae were isolated, irradiated and allowed to recover soon after irradiation; that is, irradiation (100 or 300 Gy) significantly increased the post-dauer life spans (Fig. 2a, Table1). Similar life-span extensions have been noted previously in irradiated dauer larvae.[7,18] At 500 Gy, the beneficial effect was lost.

Our approach is schematically represented in Fig. 1, as "X days" represents the time between dauer-larvae isolation and irradiation while "Y days" represents the time after irradiation before food presentation. In the first set of experiments, X was set at one day (that is, animals were irradiated one day after isolation) and the post-irradiation holding period was varied between one day and 30 days (that is, dauer larvae were held for between one day and 30 days before food presentation). Beneficial responses in terms of life span were observed under each of these conditions (Fig. 2a–2d; Fig. 3a; Table 1). Moreover, this response was neither intensified nor diminished with post-irradiation incubation.

In a second set of experiments, the time between dauer-larvae isolation (X days) was varied between one and 30 days, and the post-irradiation recovery period (Y days) was

Table 1. Mean life spans ± standard deviations of post-dauer larvae. The pre- and post-irradiation times are as given in Fig. 2a.

Pre- (X):Post-(Y)	Irradiation Doses (Gy)			
Irradiation time (days)	0	100	300	500
X = 1:Y				
Y = 1	13.2 ± 2.6	17.7 ± 4.6	16.6 ± 3.8	14.6 ± 3.1
Y = 10	13.4 ± 2.1	17.2 ± 2.3	16.0 ± 3.6	14.4 ± 3.1
Y = 20	14.2 ± 2.6	18.0 ± 4.5	16.9 ± 4.0	14.1 ± 2.6
Y = 30	13.2 ± 1.8	17.7 ± 4.3	16.7 ± 3.2	14.3 ± 3.3
X:Y = 1				
X = 1	13.2 ± 2.6	17.7 ± 4.6	16.6 ± 3.8	14.6 ± 3.1
X = 10	13.4 ± 2.1	16.7 ± 3.9	15.9 ± 3.4	14.0 ± 2.9
X = 20	14.2 ± 2.6	14.6 ± 4.5	15.3 ± 3.6	13.6 ± 3.3
X = 30	13.2 ± 1.8	12.9 ± 2.3	14.6 ± 3.2	
X = 10:Y				
Y = 1	13.3 ± 2.1	16.7 ± 3.9	15.9 ± 3.4	14.0 ± 2.9
Y = 5	13.5 ± 2.1	13.2 ± 2.7	13.0 ± 2.7	12.3 ± 2.7

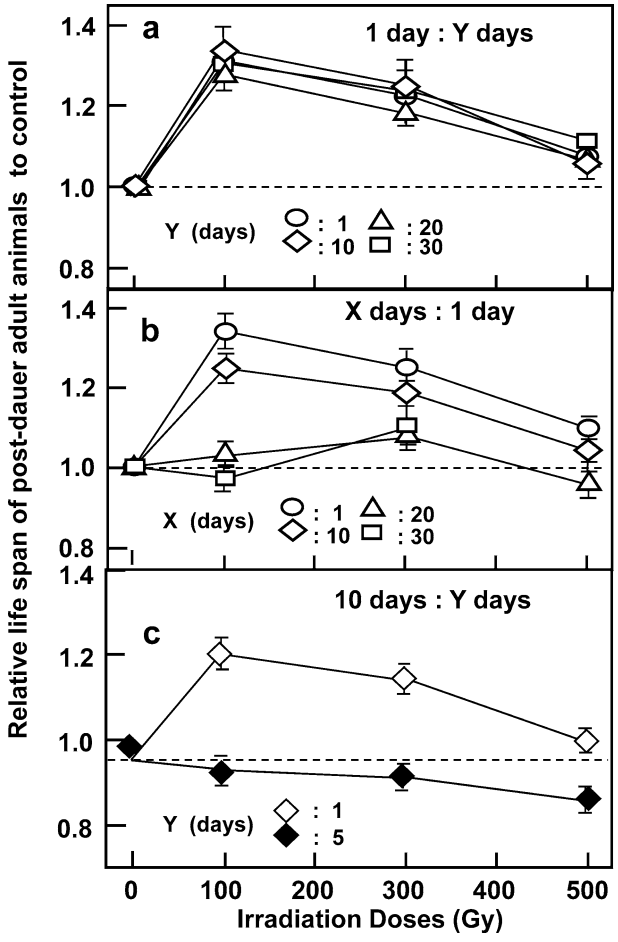

Fig. 3. Life spans of post-dauer larvae. **a**: Relative life spans when dauers were irradiated one day after isolation and held for various times before resumption of development. **b**: Relative life spans when dauers were incubated for various times of pre-irradiation and allowed to resume development one day post-irradiation; **c**: Relative life spans when dauer larvae were held for 10 days pre-irradiation and incubated an additional one or five days post-irradiation. In panels a–c, the mean life spans ± standard deviations of post-dauer larvae were compared to those of non-irradiated dauer larvae. Each point indicates the mean from three separate experiments.

fixed at one day. Unlike with the first set of experiments, the beneficial effect was lost when larvae were incubated for long periods before irradiation (Fig. 2a, 2e–2g; Fig. 3b; Table 1).

In a third set of experiments, larvae were held for 10 days before irradiation and allowed to recover after either one or five days (Fig. 2h; Fig. 3c; Table 1). Under these conditions, the post-irradiation incubation did modulate the beneficial effect; that is, dauer larvae held for one day before food presentation showed a strong beneficial effect while there was absolutely no beneficial effect observed after dauer were held for five days after irradiation. This is curious, as the first set of experiments clearly demonstrated that beneficial effect was not influenced by post-irradiation in young dauer larvae.

DISCUSSION

We report here that X-irradiation (100 or 300 Gy) significantly increased post-dauer life spans (Fig. 2; Table 1). Similar life-span extensions have been noted previously in irradiated dauer larvae.[7,18] The observed beneficial effect on life span was not enhanced by holding young dauer larvae after irradiation (Fig. 3a). This suggests that the beneficial effect was not mediated by post-irradiation events that occurred in the dauer-larval developmental state. Rather, the events that mediated life-span extension began coincident with or after dauer larvae were presented with the food that prompted their resumption of development. The molecular mechanism mediating this beneficial effect such as hormesis is unknown, but may involve induction of DNA repair. However, Johnson and Hartman have demonstrated ionizing-radiation-induced hormesis in a series of radiation-sensitive strains, including the excision-repair defective *rad-3* mutant.[7] This argues against an involvement of DNA repair, although other repair pathways may mediate the effect. Alternatively and more likely, the life-span enhancement we observed could have been the result of induction of other enzymes that conferred a protective effect. In particular, some of the superoxide dismutases so critical in providing protection against oxidative stress are known to be transcriptionally regulated. Interestingly, induction of the five *C. elegans* superoxide dismutases has recently been shown to be variably dependent upon two key regulators of life span.[19] Specifically, *sod-1*, *sod-3*, and *sod-5* induction is dependent upon either *pha-4* or *daf-16*, essential transcription factors that mediate diet restriction and the insulin-like signaling pathway, respectively. Conversely, *sod-2* is induced by *pha-4* but not *daf-16* while *sod-3* is induced by *daf-16* but not *pha-4*.[19] Given that Anderson observed that SOD levels were elevated in dauer larvae, this is likely to be one of the key determinants in dauer larvae "life span".[14]

While post-irradiation holding time did not enhance or detract from the beneficial effect seen when young dauer larvae were irradiated, dauer larvae lost their potential to undergo the beneficial effect when held before irradiation (Fig. 3b). This indicates that, even though the beneficial effect was mediated through events that are initiated during or after recovery, the potential for these events was gradually lost as larvae are held in buffer. Clearly the order of events is critical. Most notably, larvae irradiated one day after isolation and held for 30 days showed a strong beneficial effect while larvae held for 30 days pre-irradiation and held only one day post-irradiation showed no absolutely beneficial effect. In both cases the total holding time before food presentation was 31 days, but the profiles are very different.

How can this apparent discrepancy be reconciled? We propose the following model, which posits that irradiation imposes two, opposing effects on dauer larvae. The first is a beneficial effect, perhaps hormetic, that, as mentioned above, is the result of changes in gene expression. The second is a toxic effect that results from the generation of oxidative damage caused by irradiation of the "age-associated pigments" that dauer larvae are known to accumulate with time.[4] In this model, when dauer larvae were irradiated shortly after collection, age pigments were not abundant and the toxic effects of irradiation were minimal relative to the life-span enhancing effects of irradiation. Consequently, the time between irradiation and recovery to adulthood would be predicted not to affect the degree of beneficial effect. This is exactly what we observed (Fig. 3a). Conversely, if dauer larvae were held for various times of pre-irradiation and then immediately allowed to recover, the amount of oxidative stress would increase as a function of pre-irradiation incubation time. This negative effect of irradiating dauers would eventually negate any beneficial effects. The net result would be that life spans would be enhanced by irradiation of young but not old larvae. Again, this prediction is borne out by the data (Fig. 3b). The reason why the beneficial effect was lost at 500 Gy could be that radiation damage is prior than the beneficial effect.

At this junction the model does not address the third experimental observation; namely, unlike the case with young larvae (Fig. 3a), post-irradiation incubation did modulate the beneficial effect in animals that were incubated for 10 days prior to irradiation (Fig. 3c). Specifically, a post-irradiation incubation period of one day resulted in significant radiation-induced beneficial effect but absolutely no life-span enhancement was observed if dauer larvae were incubated for five days before they were allowed to resume development. This seemingly conflicts with the results in Fig. 3a and 3b. To reconcile this apparent discrepancy, we propose that the *overall* beneficial capacity did decrease as dauers were held in buffer. In the case of dauers irradiated soon after isolation, this decrease did not approach the *maximum* life-span enhancement, which is about four days. Thus, the beneficial effect was not changed when young dauer larvae were irradiated and held for various times

before development resumed (Fig. 3c). Conversely, if dauer larvae were incubated for 10 days prior to irradiation such that lipofusions accumulated to an intermediate level and if they were then held an additional 5 days such that their maximum beneficial capacity was reduced, they would not have shown the same life-span extension as if they were allowed to recover immediately after irradiation. We hope to experimentally test this model in the future.

In conclusion, a significant life-span enhancement can occur in dauer larvae after exposure to ionizing radiation. This beneficial effect is mediated by events that occur during or after recovery from the dauer larval state. The capacity for life-span enhancement is lost as dauer larvae are held in buffer. These complexities were only revealed through experiments that varied the timing of pre- and post-X-irradiation incubation before recovery.

REFERENCES

1. Riddle, D. L. and Albert, P. S. (1977) Genetic and environmental regulation of dauer larva formation. In: *C. elegans II*, Eds. Riddle, D. L., Blumenthal, T. Meyer, B. J., and Priess, J. R., pp. 739–768, Cold Spring Harbor Press, New York.
2. Wadsworth, W. G. and Riddle, D. L. (1989) Developmental regulation of energy metabolism in *Caenorhabditis elegans*. Develop. Biol. **132**: 167–175.
3. Klass, M. R. and Hirsh, D. I. (1976) Nonaging developmental variant of *C. elegans.* Nature **260**: 523–525.
4. Houthoofd, K. B., Braeckman, I., Lenaerts, K., Van Eygen, S., Vanfleteren, J. R. and De Vrese, A. (2002) Ageing is reversed and metabolism is reset to young levels in recovering dauer larvae of *Caenorhabditis elegans*. Exp. Gerontol. **37**: 1015–1021.
5. Hartman, P. S. (1984) Effects of age and liquid holding on the UV-radiation sensitivities of wild-type and mutant *C. elegans* dauer larvae. Mutat. Res. **132**: 95–99.
6. Yeargers, E. (1981) Effect of gamma-radiation on dauer larvae of *C. elegans.* J. Nematol. **13**: 235–237.
7. Johnson, T. E. and Hartman, P. S. (1988) Radiation effects on life span in *Caaenorhabditis elegans.* J. Gerontol. **43**: B137–141.
8. Lithgow, G. J., White, T. M., Melov, S. and Johnson, T. E. (1995) Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA **92**: 7540–7544
9. Yanase, S., Hartman, P. S., Ito, A. and Ishii, N. (1999) Oxidative stress pretreatment increases the X-radiation resistance of the nematode *Caenorhabditis elegans.* Mutat. Res. **426**: 31–39.
10. Cypser, J. and Johnson, T. E. (2001) Hormesis extends the correlation between stress resistance and life span in long-lived mutants of *Caenorhabditis elegans*. Hum. Exp. Toxicol. **20**: 295–296.
11. Cypser, J. R. and Johnson, T. E. (2002) Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. J. Gerontol A. Biol. Sci. Med. Sci. **57**: B109–114.
12. Yashin, A. I., Cypser, J. W., Johnson, T. E., Michalski, A. I., Boyko, S. I. and Novoseltsev, V. N. (2002) Heat shock changes the heterogeneity distribution in populations of *Caenorhabditis elegans*: does it tell us anything about the biological mechanism of stress response? J. Gerontol A. Biol. Sci. Med. Sci. **57**: B83–92.
13. Olsen, A., Vantipalli, M. C. and Lithgow, G. J. (2006) Lifespan extension of *Caenorhabditis elegans* following heat treatments. Biogerontology **7**: 221–230.
14. Anderson, G. L. (1978) Responses of dauer larvae of *Caenorhabditis elegans* (Nematoda: *Rhabditidae*) to thermal stress and oxygen deprivation. Can. J. Zool. **56**: 1786–1791.
15. Honda, Y. and Honda, S. (1999) The *daf*-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. FASEB J. **13**: 1385–1393.
16. Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. Genetics **77**: 71–94.
17. Wang, W. and Kim, S. K. (2003) Global analysis of dauer gene expression in *Caenorhabditis elegans*. Development **130**: 1621–1634.
18. Yeargers, E. (1981) Effect of gamma-radiation on dauer larvae of *C. elegans.* J. Nematol. **13**: 235–237.
19. Panowski, S. H., Wolf, S., Auilaniu, H., Durieux, J. and Dillin, A. (2007) PHA-4/Foxa meiates diet-restriciton-induced longevity of *C. elegans*. Nature **447**: 550–554.

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