The overexpression of major antioxidant enzymes does not extend the lifespan of mice

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Summary
We evaluated the effect of overexpressing antioxidant enzymes on the lifespans of transgenic mice that overexpress copper zinc superoxide dismutase (CuZnSOD), catalase, or combinations of either CuZnSOD and catalase or CuZnSOD and manganese superoxide dismutase (MnSOD). Our results show that the overexpression of these major antioxidant enzymes, which are known to scavenge superoxide and hydrogen peroxide in the cytosolic and mitochondrial compartments, is insufficient to extend lifespan in mice. Key words: aging; antioxidant enzymes; transgenic and knockout mice.

The oxidative stress theory of aging offers a credible explanation of a molecular mechanism underlying the aging process. One of the most direct tests of the oxidative stress theory of aging has been to alter oxidative stress/damage and then determine how this alteration affects lifespan (e.g. to genetically manipulate the expression of antioxidant enzymes and observe the effects on lifespan). Based on studies to date, the effect of oxidative stress as a lifespan determinant has been dependent upon the type of animal model tested. Transgenic Drosophila overexpressing either CuZnSOD (Sun & Tower, 1999; Phillips et al., 2000) or MnSOD (Sun et al., 2002) have extended longevity.

Although several studies have shown that overexpression of antioxidant enzymes in mice has a protective effect against oxidative stress, with a diminished accumulation of oxidative damage in macromolecules (Muller et al., 2007), Huang et al. (2000) reported that transgenic mice overexpressing CuZnSOD (two- to five-fold increase) did not show any increase in lifespan (Huang et al., 2000). In contrast, Schriner et al. (2005) targeted catalase to mitochondria and observed a 21% extension in the lifespan of transgenic mice.

We studied the effects of CuZnSOD and catalase overexpression on lifespan, as well as the effects of combinations of CuZnSOD and catalase or CuZnSOD and MnSOD overexpression. Transgenic CuZnSOD and catalase mice were generated using large genomic segments of the human genes containing the intact genes with their endogenous promoter (Chen et al., 2003). The MnSOD transgenic mice were generated by Dr Epstein’s laboratory using the mouse Sod2 genomic fragment (Raineri et al., 2001). These mice overexpress the expected enzymes, with increases of two- to four-fold in all tissues tested and no diminution of the expression of other major antioxidant enzymes (Raineri et al., 2001; Chen et al., 2003; Mele et al., 2006). Fibroblast cell cultures derived from these mice were observed to be more resistant to oxidant stress (Mele et al., 2006; Shan et al., 2007).

Figure 1 shows the survival curves of each single or double transgenic mouse strain compared to the wild type (WT) controls. Analysis of the survival curves by the log-rank test (Andersen et al., 1993) showed no statistical differences in the survival curves between the WT mice and any of the transgenic mice. The survival data in Table 1 also show no significant differences in the mean, median, or 90% (when 90% of the mice have died) survivals of any of the transgenic strains compared to the WT mice. It should be noted that we studied cohorts of 44–54 animals, which allow us to detect a 10% change in mean survival (Liang et al., 2003). The mean and maximum survivals for the WT mice were more than 31 and 41 months (respectively), which is in the accepted range for well-maintained colonies of C57BL/6J mice and well-run longevity studies and is higher than published results for other vivaria (see Ran et al., 2007). Thus, the husbandry conditions used in this study minimize/eliminate deaths from infectious disease and other causes that can make survival data unreliable. Our data confirm the earlier study...
by Huang et al. (2000) showing that the overexpression of CuZnSOD has no effect on the lifespan of mice, but are strikingly different from what has been observed in Drosophila in which lifespan is increased when CuZnSOD is overexpressed (Sun & Tower, 1999; Phillips et al., 2000). Our data might appear to conflict with the study by Schriner et al. (2005), which showed that the overexpression of catalase increased the lifespan of transgenic mice. However, whereas Schriner et al. overexpressed catalase in mitochondria, catalase overexpression in our transgenic mouse occurred in the peroxisomes (Chen et al.,...
2003), where catalase is normally expressed (Zamocky & Koller, 1999).

Our study is the first to determine whether simultaneously overexpressing two antioxidant enzymes has an effect on the lifespan of mice. Orr & Sohal (1994) initially reported that the overexpression of CuZnSOD and catalase together increased the lifespan of Drosophila. However, Tower’s laboratory did not find a significant increase in the lifespan of Drosophila when either catalase (Sun & Tower, 1999) or MnSOD (Sun et al., 2004) was overexpressed with CuZnSOD. As shown in Fig. 1 and Table 1, overexpressing CuZnSOD and catalase or CuZnSOD and MnSOD had no effect on the lifespan of mice.

Based on the studies with Drosophila, we anticipated that we would see an increase in lifespan in one or more of the transgenic mouse models. However, our data demonstrate that overexpression of the major antioxidant enzymes that regulate oxygen metabolism in the cell, either by themselves or in combination, does not have an impact on the lifespan of mice. Therefore, these findings do not provide support for the role oxidative stress in the aging of mice.

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References

Andersen PK, Borgan O, Gill RD, Keiding N (1993) Statistical Models Based on Counting Processes. New York: Springer.

Chen X, Mele J, Giese H, Van Remmen H, Dollie ME, Steinhelper M, Richardson A, Vigg J (2003) A strategy for the ubiquitous overexpression of human catalase and CuZn superoxide dismutase genes in transgenic mice. Mech. Ageing Dev. 124, 219–227.

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand. J. Statist. 6, 65–70.

Huang TT, Carlson EJ, Gillespie AM, Shi Y, Epstein CJ (2000) Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. J. Gerontol. A Biol. Sci. Med. Sci. 55, 85–89.

Liang H, Masoro EJ, Nelson JF, Strong R, McMahan CA, Richardson A (2003) Genetic mouse models of extended lifespan. Exp. Gerontol. 38, 1353–1364.

Mele J, Van Remmen H, Vigg J, Richardson A (2006) Characterization of transgenic mice that overexpress both copper zinc superoxide dismutase and catalase. Antioxid. Redox. Signal. 8, 628–638.

Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H (2007) Trends in oxidative aging theories. Free Radic. Biol. Med. 43, 477–503.

Orr WC, Sohal RS (1994) Extension of life-span by overexpression of superoxide dismutase and catalase in Drosophila melanogaster. Science 263, 1128–1130.

Phillips JP, Parke TL, Hilliker AJ (2000) Targeted neuronal gene expression and longevity in Drosophila. Exp. Gerontol. 35, 1157–1164.

Raineri I, Carlson EJ, Gacayan R, Canna S, Oberley TD, Huang TT, Epstein CJ (2001) Strain-dependent high-level expression of a transgene for manganese superoxide dismutase is associated with growth retardation and decreased fertility. Free Radic. Biol. Med. 31, 1018–1030.

Ran Q, Liang H, Ikeno Y, Qi W, Prolla TA, Roberts LJ, 2nd, Wolf N, Van Remmen H, Richardson A (2007) Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. J. Gerontol. A Biol. Sci. Med. Sci. 62, 932–942.

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308, 1909–1911.

Shan X, Chi I, Ke Y, Luo C, Qian S, Gozal D, Liu R (2007) Manganese superoxide dismutase protects mouse cortical neurons from chronic intermittent hypoxia-mediated oxidative damage. Neurobiol. Dis. 28, 206–215.

Sun J, Folk D, Bradley TJ, Tower J (2002) Induced overexpression of mitochondrial Mn-superoxide dismutase extends the life span of adult Drosophila melanogaster. Genetics 161, 661–672.

Sun J, Molitor J, Tower J (2004) Effects of simultaneous over-expression of CuZnSOD and MnSOD on Drosophila melanogaster life span. Mech. Ageing Dev. 125, 341–349.

Sun J, Tower J (1999) FLP recombinase-mediated induction of CuZn-superoxide dismutase transgene expression can extend the life span of adult Drosophila melanogaster flies. Mol. Cell. Biol. 19, 216–228.

Wang C, Li Q, Redden DT, Weinrodch R, Allison DB (2004) Statistical methods for testing effects on ‘maximum lifespan’. Mech. Ageing Dev. 125, 629–632.

Zamocky M, Koller F (1999) Understanding the structure and function of catalases; clues from molecular evolution and in vitro mutagenesis. Prog. Biophys. Mol. Biol. 72, 19–66.