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OXIDATIVE STRESS IN HYPOPITUITARY DWARF MICE AND IN TRANSGENIC MICE OVEREXPRESSING HUMAN AND BOVINE GH

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ABSTRACT

Growth hormone (GH) stimulates metabolic activity. The purpose of this study was to examine whether it is involved in the aging process by increasing oxidative stress. Inorganic peroxides and lipid peroxides were measured in kidney and liver samples in dwarf mice that are deficient in GH, prolactin and thyrotropin and in transgenic mice that produce high levels of GH. In normal male mice, there was an increase in inorganic peroxides in the kidney with age. Levels were lower in old male dwarfs when compared with normal male mice of similar age. Unexpectedly, concentrations of inorganic peroxides were frequently lower in transgenic male and female mice expressing extra copies of GH than in normal controls. Lipid peroxide concentrations were more variable. Transgenic animals expressing bovine GH had the highest levels of lipid peroxides. In dwarfs, kidney levels were similar to those of normal mice but concentrations in the liver were more variable. This study does not indicate that the decrease in life span in transgenic mice producing high levels of GH is due to an increase of oxidative stress. Rather, it suggests that expression of extra copies of the GH gene may lead to a compensatory increase in antioxidant protection.

INTRODUCTION

Growth Hormone: Growth hormone (GH) is named for its ability to stimulate growth of the skeletal system. However, it is also an important metabolic hormone and its effects are wide spread. As an anabolic hormone, GH promotes incorporation of amino acids during protein synthesis (Scanes, 1995). It also affects additional metabolic processes. For example, GH induces mobilization of lipids from adipose tissue and increases blood glucose levels. The latter is known as an anti-insulin or diabetogenic effect (Scanes, 1995). Many of the responses to GH are mediated indirectly through the release of somatomedin, also known as insulin-like growth factor-I (IGF-1), which is stimulated by GH.

Changes in GH secretion and oxidative stress occur during the aging process. A key question is whether or not GH, with its important effects on metabolic processes, plays a role in the changes that lead to oxidative stress and cellular breakdown during the aging process.

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Free radicals and Peroxides: There is evidence that the process of aging is caused by the damage produced by free radicals during aerobic metabolism (Harman 1981). Free radicals are chemicals that have one or more unpaired electrons, and they are destructive. Most are formed in mitochondria during respiration. Free radicals can damage proteins and other macromolecules that are critical to cell function. Although these agents are produced continuously, antioxidant enzymes provide a mechanism for their removal. For example, the superoxide radical, which is formed by one-electron reduction of oxygen, is metabolized by superoxide dismutase (SOD) to H_2O_2 and oxygen. Catalase and glutathione peroxidase convert H_2O_2 to H_2O (Yu, 1994). Normally, production of free radicals is balanced by removal, but as individuals age homeostatic control declines and radicals accumulate, a condition known as oxidative stress. Cellular damage associated with aging has been reported (Sawada et al., 1992). For example, lipid peroxides (LP), which form as a result of free radical attack of membrane phospholipids (Halliwell and Gutteridge, 1984), increase as animals age (Shi et al., 1994). Such alterations may affect the membrane environment and disrupt protein function. Oxidative stress can also be determined by measuring inorganic peroxide (e.g. H_2O_2). Higher levels correlate with damage to proteins and other macromolecules.

Transgenic Mice: The effects of chronic elevations of GH have been studied in transgenic mice. Constructs of different promoters and the GH gene from different species have been used. Foreign GH is produced in different organs, such as the liver and kidney (Palmiter et al., 1982; McGrane et al., 1988). Circulating GH levels are variable, but occasionally plasma concentrations are very high, 100 fold or more compared to normal animals (Palmiter et al., 1982, Steger et al., 1993). Although they are much larger than normal mice, transgenic mice also manifest other differences. For example, life span is reduced to half that of normal mice (Rollo et al., 1996) but this also varies (Cecim et al., 1994). Transgenic mice may also experience changes in the secretion of other pituitary hormones. Steger et al., (1994) indicate that in mice expressing bGH (bovine GH), levels of the pituitary hormones FSH and LH are depressed but that PRL secretion is somewhat elevated. Also, the GH produced in mice transgenic with the human form will bind to both GH and prolactin receptors (Tsushima and Friesen, 1973). This latter

group of mice also shows a substantial increase in mammary tumors not found in transgenic mice with the bGH gene (Cecim et al., 1994).

Previously we measured levels of the superoxide radical (SOR) and LP in transgenic mice producing high levels of rat GH (Rollo et al., 1996). We observed higher concentrations of these products than in normal mice. The life span of the transgenic mice in the study was also reduced to half that of normal animals (Kajiura and Rollo, 1994). Although it is uncertain how extra GH may increase oxidative stress, high levels appear to reduce the influence of insulin on glucose uptake in adipose tissue at a site down stream from the insulin receptor. There is evidence, for example, that GH decreases the synthesis of glut 1, a glucose transporter (Scanes, 1995). In diabetes, hyperglycemia is linked to an increase in oxygen radicals and related compounds (H_2O_2) and to protein glycosylation (Wolff et al., 1987; Hunt et al., 1988; Williamson et al., 1993). In cells in which glucose levels are elevated there is an increase in the NADH/NAD^+ ratio causing a redox imbalance. This disturbance can disrupt many metabolic pathways, some of which lead to an increase in production of oxygen radicals, ROS and lipid peroxides (Williamson et al., 1993). The result is an increase in tissue damage. The kidney is one of the target organs affected by oxidative stress in diabetes (Beyer-Mears et al., 1984; Bell et al., 1999).

Dwarf Mice: The effects of insufficient GH can be studied in dwarf animals. Ames dwarf mice are deficient in this hormone (Bartke, 1979). They lack or have few somatotrophs in the pituitary gland. In addition, they are deficient in PRL and TSH (Brown-Borg et al., 1996). These mice are much smaller when they reach adulthood, and females are sterile as a result of PRL deficiency and failure of the corpus luteum to produce progesterone for supporting pregnancy. Interestingly, Ames dwarf mice live much longer than normal mice (Brown-Borg et al., 1996). The purpose of this study was to compare the impact of high and low levels of GH on oxidative stress in the mouse.

MATERIALS AND METHODS

Dwarf Mice. Ames dwarf mice were housed under standard laboratory conditions (22 C, 12-hour light:12 hour dark cycle, and free access to food and water). Dwarf animals are produced by mating normal carriers of this recessive gene in a random bred closed colony. They are deficient in the cells that produce GH, PRL and TSH in the pituitary (Bartke 1979; Sornson et al., 1996). IGF-1 levels are also extremely low (Chandrashekar and Bartke, 1993). Adults are approximately one-third the size of normal mice.

Transgenic Mice. The founder mice for this study were developed by microinjecting bovine or human structural GH gene fused to either metallothionein-1 (MT) or phosphoenolpyruvate (PEPCK) promoters into the male pronucleus of fertilized mouse ova (McGrane et al., 1988; Steger et al., 1993;). The mice used in this study were derived from single founder animals. Plasma levels of GH vary in different lines but they are elevated in the transgenics (Cecim et al 1994). The animals weigh 50-100% more and their life span is shorter (length approximately one year) than normal mice (Cecim et al. 1994; Steger et al., 1993). In the lines we used, transgenic mice with the MT promoter have lower levels of hGH (3-15 ng/ml plasma), but they have elevated IGF-I levels. They are also larger than normal mice, and they live longer than transgenic mice with the PEPCK promoter.

Lipid Peroxide Assay. Lipid peroxides were measured in microsomes prepared from kidney and liver samples. LP were determined by the thiobarbituric acid test (Uchiyama and Mihara, 1978; Sawada and Carlson, 1985), and the results are expressed as ng of malonaldehyde per µg of protein. Microsomes were prepared by homogenizing samples in 0.1M Tris/HCl (pH 8.0) and centrifuging at 17,000 g for 30 min. Samples of the supernatant were read in a spectrometer at an absorption wavelength of 535 nm. A second reading at 520 nm was subtracted form the first in order to reduce interference (Shi et al., 1994). The results were expressed on the basis of tissue protein, which was determined by the Bradford assay (Bradford, 1976).

Inorganic Peroxide Assay. Kidney and liver samples for the inorganic peroxide (IP) assay were prepared as were the samples for the LP assay. The assay is based on the procedure described by Meiattini (1985), which we have used (Shi et al., 1994). H_2O_2 is used as the standard, 4-aminophenazone-chromotropic acid is the hydrogen donor and absorption is measured at 595 nm. The results are expressed as ng H_2O_2 per mg protein.

Statistics. The results were examined for significance using one-way analysis of variance. Pairwise comparisons were performed using LSD.

RESULTS

The results show that IP levels increase with age in normal male mice (Figure 1). Levels of this reactive oxygen species, however, were much lower in 26-month-old male dwarf mice than in comparably aged normal animals. Also, in age-matched normal and dwarf females, liver IP were lower in the dwarf mice (Figure 2). Unexpectedly, we found that IP levels in transgenic mice were not higher than in normal mice. Significantly lower levels were observed in male mice expressing bGH (Figure 3) and in the liver of female mice expressing hGH and in the kidney of females expressing bGH. (Figure 4).

The lipid peroxide data appear in Tables 1 and 2. Levels were variable. Concentrations were higher in the kidney of male mice expressing bGH than in male mice expressing hGH or dwarf males (Table 1). In females expressing bGH, kidney LP was higher than for all other groups (Table 2). Liver LP levels were higher in transgenic males than in dwarfs, and levels in dwarfs were lower

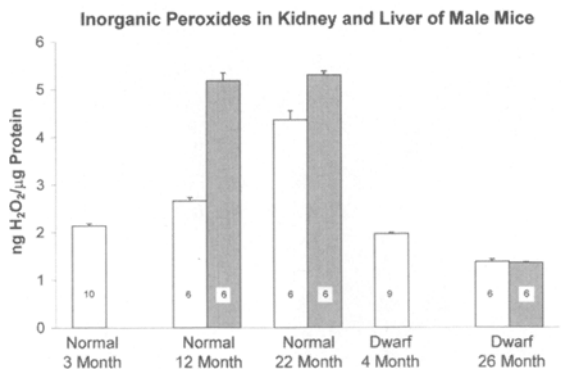

Figure 1: Inorganic peroxide concentration of kidney (open bars) and liver (stippled bars) from normal and dwarf male mice. H_2O_2 levels in kidney samples of normal 22 month mice were significantly greater (<0.01) than H_2O_2 levels in kidney samples of each of the other groups. Also, H_2O_2 in liver samples of normal 12 and 22 month mice were significantly greater (<0.01) than in liver samples of dwarf 26 month mice. Numbers within the bars indicate number of mice examined.

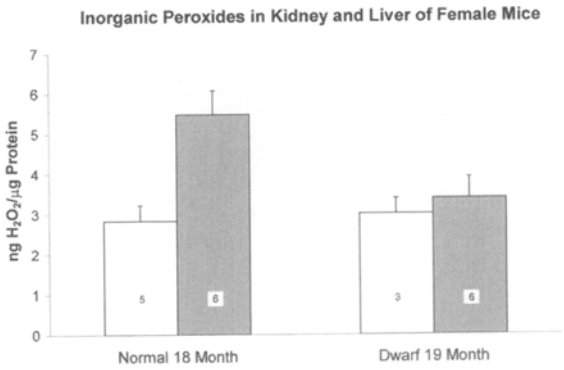

Figure 2: Inorganic peroxide concentration of kidney (open bars) and liver (stippled bars) from normal and dwarf female mice. H_2O_2 in liver samples of normal 18 month mice were significantly greater (<0.05) than in dwarf 19 month mice. Numbers within the bars indicate number of mice examined.

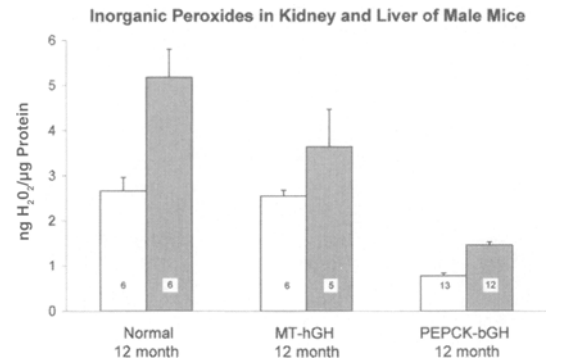

Figure 3: Inorganic peroxide concentration of kidney (open bars) and liver (stippled bars) from normal and transgenic male mice. H_2O_2 levels in kidney samples from normal 12 month and MT-hCG 12 month mice were significantly greater (<0.01) than in PEPCK-bGH 12 month mice. Also, H_2O_2 levels in liver samples were significantly different (<0.01) in each of the three groups. Numbers within the bars indicate number of mice examined.

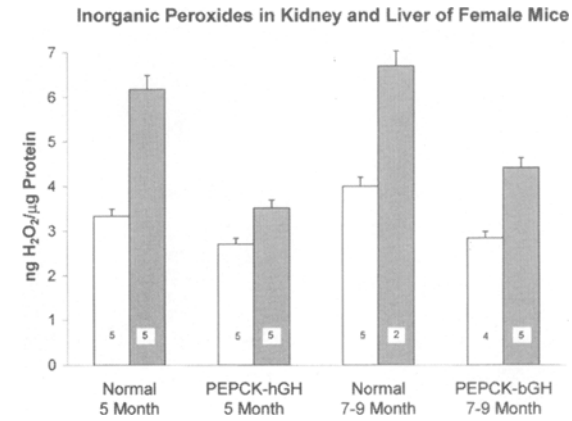

Figure 4: Inorganic peroxide concentration of kidney (open bars) and liver (stippled bars) from normal and transgenic female mice. In kidney samples H_2O_2 levels in normal 7-9 month mice were significantly greater (< 0.01) than in PEPCK-bGH 7-9 month mice. In liver samples H_2O_2 levels were significantly greater (<0.02) in normal 5 month mice than in PEPCK-hCG 5 month mice. Numbers within the bars indicate number of mice examined.

Table 1: Lipid peroxides in normal, dwarf and transgenic male mice.

		Kidney			Liver		
	Age*	Mean**	SE	n	Mean**	SE	n
Normal	12	0.282[1,3]	0.07	4	0.455	0.16	6
	22	0.307[1,3]	0.19	4	0.282	0.08	5
Dwarf	26	0.244[1]	0.05	6	<0.100	0.01	6
MT-hGH	12	0.159[1]	0.05	6	0.428	0.13	6
PEPCK-bGH	12	0.436[2,3]	0.03	13	0.406	0.04	12

*Months
**ng MDA/µg protein
[1-3]Kidney values with different numbers are significantly different

Table 2: Lipid peroxides in normal, dwarf and transgenic female mice.

		Kidney			Liver		
	Age*	Mean**	SE	n	Mean**	SE	n
Normal	5	0.251[1]	0.04	5	<0.100	0.01	5
	7-9	0.275[1]	0.05	5	0.103[1]	0.01	5
	18	0.155[1]	0.07	2	0.173[1,2]	0.01	5
Dwarf	19	0.361[1]	0.14	2	0.261[2]	0.08	6
MT-hGH	5	0.246[1]	0.06	5	0.121[1]	0.02	5
PEPCK-bGH	7-9	0.429[2]	0.03	5	0.100	0.02	5

*Months
**ng MDA/µg protein
[1-3]Kidney values with different numbers are significantly different

than in normal young males (12 months). However, in dwarf females liver LP were higher than in all groups except the 18-month old normal female mice.

DISCUSSION

The free radical hypothesis is one of the major hypotheses explaining the aging process. As proposed by Harman (1981), aging is due to the progressive increase in cellular damage caused by free radical reactions in the organism. This notion is supported by numerous studies (Pryor, 1987; Carlson and Forbes, 1992; Orr and Sohal, 1994). For example, we measured progressive increases in the superoxide radical and LP in the brain, heart and liver of aging rats. Moreover, this rise was associated with membrane damage and loss in protein synthesis (Sawada et al., 1992).

Studies with dwarf and transgenic mice exhibiting extreme levels of GH in the circulatory system present an opportunity to identify the effects of large differences in GH on changes associated with free radicals. The life span of normal mice is 2-3 years (Steger et al., 1993) and that of dwarfs, which produce little or no GH, is approximately one year longer (Brown-Borg et al., 1996). In the current study, we observed the expected age-related increase in IP in kidney samples from normal mice. Levels of IP, however, in older dwarf mice were lower. Although we are uncertain why dwarf animals exhibit greater longevity, it may be related to lower levels of metabolic activity (Bartke et al., 1998; Hunter et al., 1999) that result in reduced oxidative stress. This may be interpreted as indicating that GH, due to its stimulation of metabolic processes, is involved the aging process. However, as noted, Ames dwarf mice are also deficient in PRL and TSH (Brown-Borg et al., 1996). Although the relative impact of deficiencies of each of these three pituitary hormones is unknown, it seems that the greater longevity of the dwarfs in comparison with normal sibling mice may be linked to reduced generation of free radicals, as indicated by the free radical theory.

Previous studies with transgenic mice expressing the GH gene indicate that they grow more rapidly than normal mice and that their life span is roughly one year (Steger et al., 1993; Kajiura and Rollo, 1994). High levels of GH in the circulatory system of transgenic mice have been confirmed (Cecim et al., 1994). Studies with mice expressing the rat GH gene also reveal higher levels of SOR and LP in plasma membrane samples from kidney and liver than in samples from normal mice (Rollo et al., 1996). A higher level of oxidative stress and consequent damage, as evidenced by greater lipid peroxidation, may be causally related to the reduction of life span. In contrast to the transgenic mice expressing rat GH, the transgenics in the present study were derived from founder male mice expressing human or bovine GH. These mice produce a wide range of heterologous GH, which is also associated with reduced life span (Cecim et al., 1994). In the present study, however, the expected correlation between expression of GH in transgenic mice and high levels of inorganic peroxides in kidney and liver samples did not hold up. Contrary to the observation cited above (Rollo et al., 1996), there was either no apparent change in IP concentration or significantly less in transgenic mice. Although the explanation for this is uncertain, it seems possible that there could be higher levels of antioxidant activity in the transgenic animals. Recent studies with transgenic mice expressing hGH indicate that SOD levels in the hypothalamus are higher than in normal mice (Hauck and Bartke, 1999). Also, in rodents human GH exhibits the biological effects of PRL (Tsushima and Friesen, 1973). PRL has been reported to induce SOD activity in the rodent corpus luteum (Sugino et al., 1998), an important target organ that is critically dependent upon antioxidant activity for controlling function (Sawada and Carlson, 1996). Although the LP levels in this study were variable (Tables 1 and 2), we observed lower levels in kidney samples of mice expressing hGH than in mice expressing bGH, which does not possess PRL activity. This may be related to differences in circulating levels of GH (Cecim et al., 1994) or to the particular biological activity associated with the type of GH gene being expressed in the transgenic mouse. In general, our results indicate that transgenic mice expressing human or bovine GH do not show the anticipated increase in free radical activity. Although higher levels of GH would appear to elevate oxidative stress, it is possible that compensatory mechanisms such as increased protection by antioxidant enzymes may offset the expected damage.

ABBREVIATIONS

Growth Hormone, GH; superoxide dismutase, SOD; lipid peroxide, LP; follicle stimulating hormone, FSH; luteinizing hormone, LH; bGH, bovine growth hormone; superoxide radical, SOR; reactive oxygen species, ROS; prolactin, PRL; thyroid stimulating hormone, TSH; metallothionen, MT; phosphoenolpyruvate, PEPCK; human growth hormone, hGH; inorganic peroxides, IP; least significant difference, LSD.

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