Animal Model for Oxidative Stress Research—Catalase Mutant Mice

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Abstract

Catalase-deficient mouse strains were initially established by Feinstein et al. through a large scale screening of the progeny of irradiated C3H mice in 1966. Later, Feinstein provided the mice of catalase mutant strain C3H/AnlCs^aCs^a (wild-type), C3H/AnlCs^bCs^b and C3H/AnlCs^cCs^c to Okayama University Medical School in Japan. It is known that a point mutation at amino acid 11 (from glutamine to histidine) of acatalasemic mouse catalase and a point mutation at amino acid 439 (from asparagine to serine) of hypocatalasemic mouse catalase are responsible for the catalase deficiency of acatalasemic and hypocatalasemic mice, respectively. Recently, a liver cell line from an acatalasemic mouse and *Escherichia coli* (*E. coli*) strains with murine normal, hypocatalasemic, or acatalasemic catalase have been established. The construction of these new systems would be useful for studying the effects of oxidative stress at the cellular level. In this review, we give a brief overview of recent findings of studies in utilizing the catalase-deficient mice and evaluate the possibility of these mouse strains as a candidate animal model for oxidative stress research.

Key words: acatalasemia, catalase mutant mice, catalase activity, hydrogen peroxide, oxidative stress

Introduction

Catalase, mainly located in peroxisomes of most mammalian cells, is an important enzyme in the regulation of the intracellular hydrogen peroxide (H₂O₂) level in biological systems (1–2) by catalyzing the following reactions (3):

The mammalian catalase is a tetrameric enzyme containing 4 identical 60,000 Dalton subunits. Its molecular mass is approximately 240,000. In 1948, Takahara and Miyamoto discovered deficiencies in erythrocyte catalase among some Japanese patients who had oral gangrene (4). They named this congenital absence of erythrocyte catalase as acatalasemia, later called “Takahara’s disease” (5). Subsequently, acatalasemic patients were also found in Switzerland, Israel, USA, Peru and Hungary (6–7). In 1966, Feinstein et al. established catalase-deficient mouse strains, including acatalasemic (C3H/AnlCs^b)

and hypocatalasemic (C3H/AnlCs^c) mice, through a large scale screening of the progeny of irradiated C3H mice (8). The development of the catalase-deficient animal models has greatly helped to clarify the possible mechanism of “Takahara’s disease” among acatalasemia (5, 7). Later, Feinstein provided the mouse strains C3H/AnlCs^aCs^a (wild-type), C3H/AnlCs^bCs^b and C3H/AnlCs^cCs^c to Okayama University Medical School in Japan.

In this review, we give a brief overview of recent findings of studies in utilizing the catalase-deficient mice and evaluate the possibility of these mouse strains as a candidate animal model for oxidative stress research.

Catalase activity in mutant mice

The findings of Feinstein et al. (9) suggested that the acatalasemic mouse catalase activity is only a small fraction of the activity in normal mice: 1.75% in blood and 24.5% in the liver when measured at 37°C, and the erythrocyte catalase activity in hypocatalasemic mice was 50% of normal mice. Tottori (10) also found that the catalase activities in the lung and liver of acatalasemic mice were 69.9% and 65.7% of those in wild-type, respectively. Several studies demonstrated the instability of the acatalasemic catalase molecule under certain physical conditions, such as heat (10–11), and suggested that catalase activity in the acatalasemic mouse should be measured at 25°C. It has also been suggested that the molecular structure of mutant catalase from acatalasemic mice is more vulnerable and easier

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Table 1 Comparison of catalase activity among mutant mice*

	Normal (C3H/AnlCs ^a Cs ^a)	Hypocatalasemic (homozygous) (C3H/AnlCs ^a Cs ^a)	Acatalasemic (C3H/AnlCs ^b Cs ^b)
Hemolysate ($\mu\text{mol/sec/g Hb}$)	5.57 $\pm$ 0.95	2.34 $\pm$ 0.38	0.99 $\pm$ 0.29
Liver ($\mu\text{mol/sec/g protein}$)	34.34 $\pm$ 9.17	24.08 $\pm$ 6.53	13.59 $\pm$ 2.16
Kidney ($\mu\text{mol/sec/g protein}$)	17.67 $\pm$ 3.83	9.91 $\pm$ 3.29	2.91 $\pm$ 0.51
Heart ($\mu\text{mol/sec/g protein}$)	1.46 $\pm$ 0.47	0.56 $\pm$ 0.26	0.22 $\pm$ 0.09
Lung ($\mu\text{mol/sec/g protein}$)	3.45 $\pm$ 0.30	2.04 $\pm$ 0.86	0.98 $\pm$ 0.20
Brain ($\mu\text{mol/sec/g protein}$)	0.55 $\pm$ 0.08	0.20 $\pm$ 0.06	0.13 $\pm$ 0.02
Stomach ($\mu\text{mol/sec/g protein}$)	0.64 $\pm$ 0.05	0.26 $\pm$ 0.10	0.15 $\pm$ 0.04
Muscle ($\mu\text{mol/sec/g protein}$)	0.78 $\pm$ 0.22	0.22 $\pm$ 0.08	0.17 $\pm$ 0.06

* Catalase activities were determined spectrophotometrically at our laboratory by measuring the rate in the presence of 70 μM hydrogen peroxide at 590 nm based on the method of Masuoka et al. (14). All values are expressed as means $\pm$ standard deviation (n=3).

to be separated when compared with those from normal and hypocatalasemic mice (12). No significant difference was found in glutathione peroxidase activity in the blood between acatalasemic and normal mice (13).

Recently, a simple and sensitive method of hydrogen peroxide determination for measurement of catalase activity was developed by Masuoka et al. (14), and the catalase activities obtained spectrophotometrically by this method were in good agreement with those by the UV method. It is particularly useful when the sample is small and when many samples require analysis at the same time (12, 14). Table 1 shows a comparison of catalase activities in hemolysates and organs among 3 strains of catalase mutant mice measured at 25°C at our laboratory based on the method of Masuoka et al.

Physiological characteristics of catalase mutant mice

A study on the metabolism of ethanol and methanol showed there was no significant difference in the metabolic rate of ethanol between normal and acatalasemic mice, whereas the metabolism of methanol was decreased in acatalasemic mice (15), suggesting that catalase plays a major role in murine methanol metabolism.

Ogata et al. (16) also investigated the mercury distribution in mouse organs and blood after 10 minutes mercury vapor exposure to acatalasemic and normal mice, they found low mercury levels in the lungs and blood, higher levels in the livers and brains of acatalasemic mice compared with normal mice, and the decrease of mercury levels in the lungs of acatalasemic mice was approximately in proportion to the decrease in catalase activity, suggesting that the primary oxidative site of the inhaled mercury probably occurred in the lungs instead of the blood. They further demonstrated that the ratio of the mercury level in the brain to that of the blood of acatalasemic mice was about two folds higher than that of the normal mice. It appeared that mercury passed the blood-brain barrier more easily in acatalasemic mice than in normal mice.

Oxidant stress research

The studies by Wang et al. (17) demonstrated enhanced liver injury in the later phase (after 12 h) of carbon tetrachloride (CCl_4) intoxication in acatalasemic mice, although deprivation of hepatic iron by phlebotomy, subcutaneous injection of eryth-

ropoietin, and intraperitoneal injection of the iron-chelating agent deferrioxamine mesylate inhibited the hepatotoxicity of CCl_4 in both acatalasemic and normal mice (18). The authors concluded that the CCl_4 -induced hepatotoxicity in the later phases was probably produced through the formation of H_2O_2 or hydroxyl radicals formed from H_2O_2 in which iron is important in mediating its toxicity, and catalase plays a critical role in the prevention of CCl_4 -induced later phase hepatotoxicity.

An increase in methemoglobin formation (MetHb) was observed in the hemolysates of acatalasemic mice exposed to nitrogen oxide (NO) and nitrogen dioxide (19), however, an addition of α -tocopherol to the mouse diet appeared to be an inhibitory effect on MetHb formation in hemolysates of both acatalasemic and normal mouse groups exposed to NO (20). These results suggest the involvement of free radical reactions in the MetHb formation. The addition of catalase to hemolysates also significantly inhibited MetHb formation in acatalasemic mice, and an addition of sodium diethyldithiocarbamate, an inhibitor of superoxide dismutase activity, to normal and acatalasemic hemolysates apparently inhibited MetHb formation in both groups. The authors concluded that the decreased concentration of hydrogen peroxide would be more important than the increased concentration of superoxide anion for the reduction of MetHb formation.

Yamaoka et al. (21) reported the acatalasemic mouse brain was more sensitive to radiation: a high-dose (5.0 Gy) X-ray irradiation led to more damage to the acatalasemic mouse brain compared with the normal mice. It was reported that oxygen radicals such as hydroxyl radicals are greatly implicated with most of the harmful effects of radiation to biological systems (22). It is possible that X-ray irradiation generates OH radicals that would induce damage such as lipid peroxidation, in which H_2O_2 that could not be decomposed in acatalasemic mice was possibly produced.

Carcinogenesis prevention

A study by Ishii et al. (23) demonstrated that female acatalasemic and hypocatalasemic mice had an increased incidence of spontaneous mammary tumors. In comparison with the normal mice, the mean catalase activity of mammary glands in pregnant acatalasemic and hypocatalasemic mice was 18.8% and 49.6%, respectively. The authors further examined the effect of vitamin E on mammary tumorigenesis and found that the

cumulative incidence of spontaneous mammary tumors in vitamin E-deprived acatalasemic mice was significantly higher than that in vitamin E-supplemented acatalasemic mice, suggesting the involvement of free radicals in mammary carcinogenesis.

It was reported that acatalasemic mice were more susceptible to diethylnitrosamine (DEN), leading to enhanced hepatocarcinogenesis in comparison with normal mice (24). Raddy et al. (25) also found a higher incidence of nafenopin-induced hepatocellular carcinoma in acatalasemic mice. These results suggest H_2O_2 is involved in the processes of chemical-induced hepatocarcinogenesis and the importance of catalase in protecting the liver against H_2O_2 toxicity.

Mutation site of catalase gene

Shaffer et al. (26) discovered a point mutation at amino acid 11 (from glutamine to histidine) of acatalasemic mouse catalase gene, which was suggested to be responsible for the catalase deficiency of the acatalasemic mouse. Recently, Wang et al. (12) found a single nucleotide mutation in hypocatalasemic catalase cDNA leading to an amino acid substitution of asparagine to serine at position 439. Asparagine at this position is located on the tenth α -helix within the fourth domain of the catalase subunit, and a substitution from asparagine to serine at this position would probably affect the formation of the substrate channel leading to the heme group in the tetrameric catalase molecule, resulting in the low catalase activity observed in the hypocatalasemic mouse. With respect to the human catalase gene of acatalasemia, Wen et al. found an amino acid substitution of guanine to adenine at the fifth position of intron 4, leading to a defective catalase synthesis in Japanese acatalasemia (27).

Potential Usefulness of the animal model

Increasing numbers of chemicals are synthesized for industrial and consumer use, it is impossible to conduct long-term rodent bioassays for the detection of carcinogens in all chemicals. The Ames test, a short-term test system, has been widely employed in screening the mutagenic potential of new

chemicals. Its predictivity of a positive mutagenic response for rodent carcinogenicity was greater than 70% (28). New approaches are required to compensate for the inadequacies of the Ames test in detecting potential chemical mutagens (carcinogens). Presently, there has been an increasing awareness that the cellular formation of highly reactive oxygen species (ROS) such as H_2O_2 or hydroxyl radicals probably cause the DNA damage that finally contributes to carcinogenesis (2). Catalase is an important component of the cellular defense system against damage induced by ROS. In terms of the regulation of intracellular H_2O_2 in biological systems, both catalase and glutathione peroxidase are responsible, the former was suggested to play a major role in H_2O_2 breakdown, particularly when H_2O_2 is overproduced (29). The reactive hydroxyl radicals, produced from H_2O_2 by the Fenton reaction or the Haber-Weiss reaction in the presence of transition metals, are capable of causing oxidative DNA damage (30–31). Kondo et al. (32) established liver cell lines with very low catalase activity from an acatalasemic mice. They proposed that this cell line can be used to detect the low oxidative stress that cells with normal catalase activity can not, and they finally concluded the catalase-deficient hepatocyte cell line would be useful for studying the effects of oxidative stress at the cellular level. Recently, mutant catalase cDNAs from the hypocatalasemic and acatalasemic mice were also successfully cloned and expressed in *E. coli*. (12). The construction of catalase mutant *E. coli* strains raises a question whether these strains would be useful for the screening of mutations resulting from oxidative stress due to chemicals. According to plenty of research data on catalase mutant mice, either the catalase mutant mice themselves or other types of systems (catalase mutant *E. coli* or cell lines) appear to have usefulness as screening models for hazard identification of oxidative chemicals in a risk assessment process. We believe this mouse model would have potential to provide new information regarding the role of oxidative stress in future investigations.

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