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Abstract

Some mechanisms to reduce methemoglobin (metHb) formation for the maintenance of normal oxygen transport have been proposed. To study the role of catalase (EC 1.11.1.6), metHb formation in the hemolysate of normal and Japanese acatalasemic human subjects were examined spectrophotometrically. Significantly increased level of metHb was induced by potassium ferrocyanide in the hemolysate of acatalasemic subject. The addition of catalase reduced the metHb formation, while 3-amino-1,2,4-triazole (AT), a specific inhibitor of catalase-H2O2 compound I, increased it. These results obtained from human subjects were well consistent with those from mice and suggested that catalase plays a role in protecting erythrocytes against metHb formation.

KEYWORDS: methemoglobin, catalase, acatalasemia, potassium ferrocyanide, biological monitoring

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The Role of Catalase in Protecting Erythrocytes against Methemoglobin Formation

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Some mechanisms to reduce methemoglobin (metHb) formation for the maintenance of normal oxygen transport have been proposed. To study the role of catalase (EC 1.11.1.6), metHb formation in the hemolysate of normal and Japanese acatalasemic human subjects were examined spectrophotometrically. Significantly increased level of metHb was induced by potassium ferrocyanide in the hemolysate of acatalasemic subject. The addition of catalase reduced the metHb formation, while 3-amino-1,2,4-triazole (AT), a specific inhibitor of catalase-H_2O_2 compound I, increased it. These results obtained from human subjects were well consistent with those from mice and suggested that catalase plays a role in protecting erythrocytes against metHb formation.

Key words : **methemoglobin, catalase, acatalasemia, potassium ferrocyanide, biological monitoring**

Acatalasemia is a congenital constitutional abnormality characterized by the lack of catalase and was first described by Takahara in 1948 (1). Catalase (EC 1.11.1.6) is one of the most active catalysts produced by nature, decomposing hydrogen peroxide. Some mechanisms to reduce metHb formation for the maintenance of normal oxygen transport have been proposed (2, 3). Some studies (4, 5) have suggested that catalase inhibits the formation of metHb. In the previous study (6), we demonstrated the inhibitory effect of catalase on metHb formation, using the hemolysate of acatalasemic, hypocatalasemic and normal mice. Thus acatalasemia and hypocatalasemia were considered to be hypersusceptible to metHb inducers.

In the present study, we examined spectrophotometrically metHb formation in the hemolysate of normal and acatalasemic human subjects, using potassium ferrocyanide as the reagent for metHb induction.

Materials and Methods

Human blood. Blood samples were taken from the antecubital vein of three normal subjects and a Japanese acatalasemic subject, whose genotypic and phenotypic characterization of biochemical defect had been throughly studied.

Reagents. Potassium ferrocyanide [$K_4Fe(CN)_6$] was obtained from Wako Pure Chemical Ind., Osaka, Japan. Bovine liver catalase (42,000 Sigma Units/mg of protein) was obtained from Sigma Chemical Co., St.

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Louis, MO, USA. 3-Amino-1,2,4-triazole was obtained from Tokyo Kasei Co., Tokyo, Japan. All the reagents were of analytical grade or equivalent.

Assay of catalase activity. Catalase activity was determined at 20°C, according to the perborate method of Feinstein (1949), which was expressed in perborate units (PU/gHb) (7).

Preparation of hemolysate. Blood samples were centrifuged and the sediments were washed three times with the physiological saline. Three volumes of distilled water was added to the sediments to hemolyze erythrocytes and then centrifuged at 10,000 rpm for 60 min at 0°C. The supernatant was diluted with 0.1 M Na, K-phosphate buffer (pH 6.8) to adjust the hemoglobin concentration to 0.6 mg/ml.

MetHb induction. Hemolysate, thus obtained, was placed in a glass cuvett with a light path of 1-cm and then incubated in air at 37°C without shaking. The reaction was initiated by the addition of 8 or 16 mM final concentration of potassium ferrocyanide.

MetHb formed was measured at various time intervals (5, 10, 15, 20 and 30 min), with a Hitachi model 100-50 spectrophotometer at 541 and 630 nm according to the modified Van Kampen method (8). MetHb level was expressed as percentage of metHb to total hemoglobin [(metHb/total Hb) × 100].

To examine the effect of catalase on metHb formation, catalase was added to the hemolysate of acatalasemic subject before initiating the reaction with potassium ferrocyanide. 3-Amino-1,2,4-triazole (AT) was added to the hemolysate of normal subjects in the same way to certify the generation of H_2O_2 in the reaction. Final concentration of the reagents were: catalase, 1.6 PU/ml; AT, 40 mM.

Statistical study. The difference between the means of two independent groups was tested for statistical significance. Student's *t*-test was applied when variances of two populations were assumed to be equal, while the Welch test was used in cases where they were different.

Results

Catalase activity. Catalase activities (mean ± SD) in the hemolysates of three normal subjects averaged 3,033.5 ± 220.1 PU/gHb and three different samples from a Japanese acatalasemic subject averaged 4.1 ± 1.2 PU/gHb (0.14 % of the normal), respectively.

MetHb formation by potassium ferrocyanide. Effects of potassium ferrocyanide on metHb formation in the hemolysates of normal and acatalasemic subjects are shown in Table 1. At and after 5 min significantly increased level of metHb was formed by 8 and 16 mM potassium ferrocyanide in the hemolysate of acatalasemic subject ($p < 0.05$; examined by *t*-test).

Effect of catalase on metHb formation. As indicated in Table 2, addition of catalase to the hemolysate of Japanese acatalasemia significantly reduced the metHb formation by potassium ferrocyanide ($p < 0.05$; *t*-test). The degree of inhi-

Tabele 1 Methemoglobin formation in the hemolysate of normal and Japanese acatalasemic subjects at various time intervals in the presence of various concentrations of potassium ferrocyanide at 37°C.

Conc. of $K_4Fe(CN)_6$ (mM)	Subject[a]	Methemoglobin level (%)[b]				
		5 min	10 min	15 min	20 min	30 min
None	N	0.56±0.11	0.83±0.21	1.28±0.26	1.47±0.41	1.62±0.38
	A	0.72±0.17	0.96±0.24	1.27±0.31	1.56±0.35	1.81±0.43
8	N	5.40±0.90	8.10±0.95	10.90±0.87	14.13±1.06	20.40±1.73
	A	8.43±1.27*	16.50±1.51**	24.57±0.64**	32.27±1.37**	43.63±2.54**
16	N	8.10±0.83	13.50±0.90	19.20±0.95	22.80±1.04	32.70±1.87
	A	13.90±1.30**	26.77±2.16**	37.10±1.73**	43.63±1.68**	51.87±1.93**

a: N means normal and A means acatalasemic Japanese subject.

b: Values are expressed as mean ± SD of three subjects (N) or three samples (A).

The difference of means between normal and acatalasemic subjects are certified by Student's *t*-test. *$p < 0.05$; **$p < 0.01$

Table 2 Effect of catalase on methemoglobin (metHb) formation in the hemolysate of Japanese acatalasemic subject by potassium ferrocyanide[a] at 37°C.

Additions[b]	Catalase activity (PU/gHb)	MetHb level (%)[c] 10 mis	20 min	30 min
None	4.1	16.50±1.51	32.27±1.37	43.63±2.54
Catalase (50μl)	1,317.6	12.47±0.64	20.53±1.58	25.30±1.31
Catalase (100μl)	2,631.2	9.90±1.10	17.23±1.27	22.00±1.63

a: Final concentration of $K_4Fe(CN)_6$ was 8 mM.
b: 50 PU/ml of catalase was added.
c: Values are expressed as mean ± SD of three samples.
The difference of means between each two independent groups is certified by Student's *t*-test. * $p < 0.05$; ** $p < 0.01$

Table 3 Effect of 3-amino-1, 2, 4-triazole (AT) on methemoglobin (metHb) formation in the hemolysate of normal subjects by potassium ferrocyanide[a] at 37°C.

Additions[b]	MetHb level (%)[c] 10 min	20 min	30 min
None	8.10±0.95	14.13±1.06	20.40±1.83
AT	15.03±1.53**	27.47±0.95**	40.53±1.61**

a: Final concentration of $K_4Fe(CN)_6$ was 8 mM.
b: 40 mM Final concentration of AT was added.
c: Values are expressed as mean ± SD of three subjects.
The difference of means between with and without AT is certified by Student's *t*-test. ** $p < 0.01$

bition depended on the dose of catalase added to the hemolysate.

Effect of AT. As shown in Table 3, significantly increased level of metHb were induced in the hemolysate of normal subjects in the presence of AT ($p < 0.01$; *t*-test). Catalase activity (mean ± SD) in the hemolysate of normal subjects decreased from 3,033.5 ± 220.1 PU/gHb to 52.3 ± 10.4 PU/gHb (1.7 % of the control) 30 min after the addition of AT.

Discussion

Some of the industrial chemicals and drugs are assumed to induce metHb formation. Aniline derivatives, nitrobenzene and nitrite are well-known metHb inducers (9). Increased metHb formation is harmful to the normal oxygen transport. There are some mechanisms to reduce metHb level in the erythrocyte. NADH methemoglobin reductase (EC 1.6.99.3), reduced glutathione and ascorbate play roles in reducing metHb to deoxyHb (2). In addition, catalase, glutathione peroxidase (EC 1.11.1.9) and superoxide dismutase (EC 1.15.1.1) are assumed to inhibit metHb formation by decomposing oxidants such as hydrogen peroxide (3, 4). Thus patients deficient in these enzymes might suffer from methemoglobinemia due to hypersensitivity to metHb inducers (5, 10).

As reported previously, catalase plays an important role in the protection against metHb formation in the hemolysate of mice (6). In the present study, we examined spectrophotometrically the effect of catalase against metHb formation using the hemolysate of normal and acatalasemic Japanese subjects. MetHb formation in the hemolysate of acatalasemic subject was significantly higher than the normal. The addition of catalase reduced the formation of metHb. In contrast, significantly increased level of metHb was induced in the hemolysate of normal subjects treated with AT, which is an irreversible inhibitor of catalase-H_2O_2 compound I. This effect of AT is an evidence of H_2O_2 generation in the reaction. These results obtained from human subjects were

well consistent with those from mice (6). It also supports a mechanism of the reaction of HbO_2 with potassium ferrocyanide proposed by Kawanishi and Caughey (4), in which H_2O_2 as well as metHb is generated by one electron transfer to O_2 from both ferrocyanide iron (II) and heme iron (II). It is well suggested by the present study that hydrogen peroxide, which also promotes metHb formation, is generated in the process of metHb induction by potassium ferrocyanide, and that catalase inhibits it by decomposing the generated H_2O_2.

Our results obtained from a Japanese acatalasemic subject suggested that acatalasemia and hypocatalasemia are susceptible to methemoglobinemia. Thus such workers should be protected from metHb inducers in the workplace (11).

References

1. Takahara S and Miyamoto H: Clinical and experimental studies on the odontogenous progressive necrotic ostitis due to lack of blood catalase. J Otorhino-Laryngol Soc Jpn (1948) **51**, 163–164 (in Japanese).
2. Scott EM, Duncan IW and Ekstrand V: Reduction of methemoglobin. Fed Proc (1963) **22**, 467.
3. Noda Y, Narumiya S, Takai K and Hayaishi O: Superoxide dismutase activity in acatalasemic erythrocytes; in Biological and Medical Aspect of Active Oxygen, Hayaishi and Asada eds, University of Tokyo Press, Tokyo (1977) pp293–297.
4. Kawanishi S and Caughey WS: Mechanism of electron tranfer to coordinated dioxgen of oxyhemoglobins to yield peroxide and methemoglobin. J Biol Chem (1985) **260**, 4622–4631.
5. Ogata M, Ishii K, Ioku N and Meguro T: Methemoglobin formation in the blood of Japanese subjects and mice suffering from acatalasemia in response to methemoglobin inducers. Physiol Chem Phys Med NMR (1990) **22**, 125–134.
6. Ohmori T and Ogata M: Methemoglobin formation by potassium ferrocyanide in the hemolysate of acatalasemic mice. Acta Med Okayama (1991) **45**, 209–215.
7. Feinstein RN: Perborate as substrate in a new assay of catalase. J Biol Chem (1949) **180**, 1197–1202.
8. Van Assendelft OW: Spectrophotometry of Haemoglobin Derivatives. Royal Vangorcum Ltd., Assen Netherland (1970).
9. Smith RN, Alikaitis AA and Shafer PR: Chemically induced methemoglobinemia in the mouse. Biochem Pharmac (1967) **16**, 317–328.
10. Schwarts JM and Jaffe ER: Hereditary methemoglobinemia with defficiecy of NADH dehydrogenase; in The Metabolic Basis of Inherited Disease, Standury *et al.* eds, McGraw-Hill Book Comp., New York (1978) pp1452–1464.
11. Methemoglobin inducers: Documentation of Biological Exposure Indices, Vera Thomas *et al.* eds, ACGIH, Ohio (1989).

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