Free Rad. Res. Comms., Vols. 12-13, pp. 669-673
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PLASMA SUPEROXIDE DISMUTASE MEASUREMENT IN CHILDREN WITH VIRAL HEPATITIS

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Seventy-seven blood samples from normal controls aged 0–8 years and 93 blood samples from children of similar ages with various viral hepatitis were investigated by measuring plasma superoxide dismutase (EC 1.15.1.1) using chemiluminescence immunoassay (CLIA). Total and Cu,Zn-SOD activities of normal controls of group 2 (1–8 years old) were significantly higher than that of normal controls of group 1 (0–1 year old) ($P < 0.01$, $P < 0.01$), while there were no differences of Mn-SOD activities between the two groups. Total, Cu,Zn- and Mn-SOD activities significantly increased in the acute phase (0–4 weeks after onset) and dropped to the normal levels in the restoration phase (4th week later) for 29 children with cytomegalovirus hepatitis (CMVH), in comparison with group 1. Only Mn-SOD activities were significantly increased in the acute phase (with increased ALT levels) and restoration phase (with normal ALT levels) for 18 children with hepatitis A (HA). Total and Cu,Zn-SOD activities significantly decreased and Mn-SOD activities significantly increased in both the active (with increased ALT levels) and the inactive phases (with normal ALT levels) for 36 children with chronic persistent hepatitis (CPH). Only Cu,Zn-SOD activities fell significantly in both active and inactive phases for 10 children with chronic active hepatitis (CAH).

KEY WORDS: Superoxide dismutase, chemiluminescence immunoassay, viral hepatitis, children.

INTRODUCTION

Since superoxide dismutase (EC 1.15.1.1) was discovered by McCord and Fridovich in 1969, many studies on it have been carried out. At present, 3 types of SOD are known, Cu,Zn-SOD, Mn-SOD and Fe-SOD. In mammals, only Cu,Zn-SOD and Mn-SOD can be found. Cu,Zn-SOD occurs in the cytosol fraction and Mn-SOD in the mitochondria.[1] All 3 types of SOD function to dismute the superoxide anion ($O_2^{\cdot -}$) at a rate of $2 \times 10^9 M^{-1} S^{-1}$.[2,3] Thus SOD protects the body from damage caused by oxygen radicals. Liver is one of the organs rich in SOD. After hepatocellular damage, it might be expected that SOD would be released into plasma and thus provide a sensitive index of hepatocellular integrity.

In recent years many studies on the SOD activities of patients with viral hepatitis, hepatic cirrhosis, chemical liver damage, alcoholic liver damage and primary liver cancer (3–7) have been carried out. In the present paper, we have measured the changes in SOD activities in children with viral hepatitis, using chemiluminescence immunoassay (CLIA). We have examined the sensitivity of the SOD activity as an index of liver damage caused by viral infection.

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MATERIALS AND METHODS

Reagents

Xanthine was purchased from Sigma Chemical Company St. Louis MO, U.S.A. luminol was the products of Merck-Schuchardt. All chemicals were of analytical grade and commercially available.

Light emission assay

The total volume of the reactive system was 1 ml or more including 500 μl xanthine (2×10^{-4} M) with or without KCN (4 mM), and 500 μl of luminol (2×10^{-4} M) with xanthine oxidase (1.8 μl/ml). 10–20 μl blood samples were used in the assay. Luminescence was monitored with a luminometer (WDD-I Beijing) at 20°C. The SOD activities were calculated by measuring inhibition of luminescence.

The Cu,Zn-SOD activities could be completely inhibited by KCN (4 mM), Mn-SOD activities were calculated by substraction of Cu,Zn-SOD activities from the total SOD activites.

Human Blood Samples

Seventy-seven blood samples of normal controls aged 0–8 years were obtained from a kindergarten in Beijing. All normal controls were free of diseases of heart, liver or kidney and had no acute infections, drug administration or hypoxaemia. All hepatic functional results including TTT, ALT, AST, and bilirubin level were normal. The viral markers including anti-HAV-IgM, HBsAg, anti-EBV-IgM and anti-CMV-IgM were confirmed as negative in the controls.

Ninety three blood samples from patients hospitalized in our unit were studied. The diagnosis of the patients with cytomegalovirus hepatitis (CMVH) was made according to the conditions as follows: a. pathological jaundice: b. hepatosplenomegaly accompanied by abnormal hepatic functional results: c. positivitiy of plasma anti-CMV-IgM.

All blood samples from the normal controls and the patients were obtained by vein punctures in the early morning before breakfast. 2–3 ml blood was stored at room temperature for 20 min and then centrifuged at 1,200 rpm. The plasma was divided into two aliquots. One was immediately examined for hepatic functional parameters and viral markers. The other was stored at −20°C for measurement of SOD activities.

Treatment of Results

All results were shown as mean ± SD. X-test and Student's t-test were used to test significance.

RESULTS

SOD Activities for Normal Controls

The normal controls were divided into two groups, group 2 (1–8 years old) and group 1 (0–1 year). Total and Cu,Zn-SOD activities in plasma of group 2 were significantly higher than those of group 1, but Mn-SOD activities were the same (Table I).

TABLE I
SOD activities for normal controls (U/ml)

	group 1			group 2		
	No.	Mean	SD	No.	Mean	SD
total SOD	40	188.34	101.84	34	237.40	133.35**
Mn-SOD	26	32.44	19.80	37	32.50	17.45
Cu,Zn-SOD	29	89.44	62.67	34	212.94	120.13**

**significant to group 1, $P < 0.01$

SOD Activities for Children with CMVH

All children with CMVH were less than 1 year old. Total, Cu,Zn- and Mn-SOD activities were increased in the acute phase (0–4 weeks after onset) and dropped to the normal levels in the restoration phase (4th week later) for 29 children with cytomegalovirus hepatitis (CMVH), comparing with normal controls of group 1 (Table II).

TABLE II
SOD activities for patients with CMVH (U/ml)

	group 1			acute phase			restoration phase		
	No.	mean	SD	No.	mean	SD	No.	mean	SD
total SOD	40	188.34	101.84	21	266.80	171.60*	9	194.74	116.54
Mn-SOD	26	32.44	19.80	22	114.10	115.2**#	9	60.75	53.00
Cu,ZN-SOD	29	89.44	62.67	29	146.52	89.15**	9	133.99	121.53

*significant to group 1, $P < 0.05$
**significant to group 1, $P < 0.01$
#significant to restoration phase, $P < 0.01$

SOD Activities for Children with HA

Mn-SOD activities were increased in both the acute phase (with abnormal ALT levels) and the restoration phase (with normal ALT levels) for 18 children with hepatitis A (HA). Total and Cu,Zn-SOD activities remain unchanged (Table III).

TABLE III
SOD activities for patients with HA (U/ml)

	group 2			acute phase			restoration phase		
	No.	mean	SD	No.	mean	SD	No.	mean	SD
total SOD	34	237.40	133.35	18	270.67	217.81	10	204.32	166.82
Mn-SOD	37	32.50	17.45	18	90.28	74.59**	10	60.51	39.19*
Cu,Zn-SOD	34	212.94	120.13	18	180.39	191.50	10	156.47	153.18

*significant to group 2, $P < 0.05$
**significant to group 2, $P < 0.01$

SOD Activities for Children with CPH

Total SOD activities were significantly lower in both the active phase (with abnormal ALT levels) and the inactive phase (with normal ALT levels) and so were the

Cu,Zn-SOD activities for 36 chilren with chronic persistent hepatitis (CPH). But Mn-SOD activities were increased in both active and inactive phases (Table IV).

TABLE IV
SOD activities for patients with CPH (U/ml)

	group 2			active phase			inactive phase		
	No.	mean	SD	No.	mean	SD	No.	mean	SD
total SOD	34	237.40	133.35	36	174.33	96.54*	15	168.99	70.70*
Mn-SOD	37	342.50	17.45	36	66.64	54.89**	15	59.70	35.33**
Cu,Zn-SOD	34	212.94	120.13	36	107.75	91.92**	15	109.20	58.73**

*significant to group 2, $P < 0.05$
**significant to group 2, $P < 0.01$

SOD Activities for Children with CAH

Only Cu,Zn-SOD activities were significantly decreased in both the active and the inactive phases for 10 children with chronic active hepatitis (CAH) comparing with the normal controls of group 2. Total and Mn-SOD activities were not significantly changed (Table V).

TABLE V
SOD activities for patients with CAH (U/ml)

	group 2			active phase			inactive phase		
	No.	mean	SD	No.	mean	SD	No.	mean	SD
total SOD	34	237.40	133.35	10	194.83	130.86	9	167.57	104.84
Mn-SOD	37	32.50	17.45	10	60.01	50.78	9	51.71	47.97
Cu,Zn-SOD	34	212.94	120.13	10	123.11	122.85*	9	115.86	62.30**

*significant to group 2, $P < 0.05$
**significant to group 2, $P < 0.01$

Relations between SOD Activities and Liver Functional Results

SOD activities and TTT, ALT, and bilirubin levels were measured for each blood sample. There were no correlations between the SOD activities and liver functional results for any of the patients with viral hepatitis. SOD activities did not better reflect the liver damage caused by the viral infections than other liver functional results.

SOD Activities and Viral Markers

According to the state of plasma HBeAg, the patients with CPH and CAH could be divided into two groups. There were no differences of SOD activities between the patients with plasma HBeAg and the patients without plasma HBeAg.

DISCUSSION

Seventy-seven blood samples from normal controls were measured for their total,

Cu,Zn- and Mn-SOD activities. The results indicated that there were significat differences of total and Cu,Zn-SOD activities in groups of different ages. So the difference of the ages should be considered when a study is carried out on children.

Plasma Mn-SOD activities were increased in patients with CMVH, CPH and HA, but remain unchanged in patients with CAH.

Plasma Cu,Zn-SOD activities were increased in patients with CMVH, remained unchanged in patients with HA, but decreased in patients with CPH and CAH. The changes of total SOD activities were similar to those of Cu,Zn-SOD because Cu,Zn-SOD was the main fraction of total SOD activity.

Japanese scientists have demonstrated that Mn-SOD activities are increased in the acute phase of patients with acute viral hepatitis and active phase of the patients with chronic viral hepatitis.

In the light of our experimental results and the literature we divide the changes of SOD activity into two types.

Type 1. SOD is released into serum when the liver cells are damaged by chemical agents, viral infections or trauma. So the SOD activity change of type 1 is mainly based on liver cell damages. Thus the plasma SOD could reflect the degrees of damage. In the present paper, the changes of SOD activity for patients with CMVH and HA belong to this type, as were liver damage by alcohol (6)

Type 2. Type 2 change of SOD activities was more complicated. The changes of this type were not mainly caused by damage of liver cells, but by the production and regulation of SOD under various pathological conditions. The changes of SOD activities for patients with CPH and CAH belong to type 2. Cu,Zn-SOD activities were decreased in patients with CAH and CPH, indicating Cu,Zn-SOD was not induced in these patients. The mechanism of production and regulation of SOD in patients with chronic hepatitis is still under investigation in our hospital.

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Accepted by Prof. G. Czapski