HSP_{60}, HSP_{70} in the Pathogenesis of Kawasaki Disease: Implication and Action

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Summary: HSP_{60}, HSP_{70} in plasma of 11 cases of Kawasaki diseases (KD) and 23 healthy children were determined. The two groups were controlled for age. Determination of HSP_{60}, HSP_{70} was conducted in lymphocytes of 14 cases of KD and 26 healthy children. The results were compared with those of 12 patients with febrile diseases and 10 patients with tuberculosis. Our results showed that except a significant difference in plasma HSP_{70} found between acute phase and convalescent phase of KD ($P<0.01$), no significant difference was found in HSP_{60}, HSP_{70} among all groups ($P>0.05$). The differences in HSP_{60}, HSP_{70} in lymphocytes were relatively obvious among all groups. The levels of HSP_{60}, HSP_{70} in acute phase of KD were significantly higher than those in convalescent phase or in healthy controls ($P<0.01$). The levels of HSP_{60} in KD were significantly higher than those of patients with febrile diseases. HSP_{60} of KD children was significantly lower than those of children with tuberculosis ($P<0.01$). The findings showed that HSP_{60}, HSP_{70} might contribute to the pathogenesis of KD. Determination of HSP_{60}, HSP_{70} in lymphocytes is of help in the diagnosis of KD.
Key words: heat shock protein; Kawasaki disease; pathogenesis

The pathogenesis of Kawasaki disease (KD) has not been fully clarified. Its clinical manifestations and epidemic features suggest that it may be associated with infection but such hypothesis has not been supported by laboratory or experimental evidences. At present, the diagnosis of the disease is mainly based on symptoms and physical signs. The aim of the study is to explore the role of heat shock protein-60 (HSP_{60}), HSP_{70} in the pathogenesis of KD.

1 MATERIALS AND METHODS

1.1 Experimental Groups

1.1.1 KD Group KD group consisted of 14 cases, among them 12 were males and 2 were females and their average age was 2.6 years. Age of 13 cases ranged from 11 months to 4 years and 1 case was 8 years old. Diagnosis was made according to the criteria established by the Japanese Kawasaki Disease Research Committee[1]. All of the venous blood samples were taken 5-10 day after fever onset when patients temperature reached 39.5±3 ℃, and before the commencement of treatment with intravenous gammaglobin. The leukocytes were isolated with density gradient centrifugation using Mono-Poly Resolving Medium.

1.1.2 Febrile Disease Group Febrile disease group had 12 cases, including 7 males and 5 females. Their age was between 1-5 years with average being 3 years. The diseases included respiratory infections ($n=7$), acute bacterial dysentery ($n=1$), hyperpyretic convulsion ($n=1$), infectious mononucleosis ($n=2$), lower pathogenic bacterial septicemia ($n=1$). All the venous blood samples were collected when patients' temperature was higher than 39 ℃.

1.1.3 Tuberculosis Group This group included 10 cases, and the age of them were all under 5 years. Among them, 8 were males and 2 were females. They consisted of 5 cases of tubercular meningitis, 3 cases of tubercular pleurisy, 1 case of pulmonary tuberculosis, and 1 case of osteoarticular tuberculosis.

1.1.4 Control Group Twenty-six healthy children aged between 3-5 years served as normal controls. Among them, 15 were males and 11 were females. They had not suffered from any recent infectious or other

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diseases.

1.2 Methods

The HSP_{60}, HSP_{60} levels were determined by the methods of modified Western dot Blot.

1.2.1 Determination of Plasma HSP_{60}, HSP_{70} Plasma sample (40 μl) was mixed with 160 ml phosphate buffer (pH=7.4). Then sample-buffer of equal volume was added. NC membrane, cut as desired, was mounted on the spot-sampling device. The diluted sample-buffer of 40 μl/well was separately added. The prepared NC membrane mixed with 5 % skimmed milk powder was put in a dish and subjected to vibration for 1 h at the temperature of 37 ℃. After blockage, the NC membrane was washed with PBS containing 0.05 % Tween 20 for 10 min×6 times. The NC membranes were put into blockading liquid containing rabbit to human HSPs (Robert M, Tanguay, Quebec, Canada), vibrated at 37 ℃ for 2 h. Again, the PBS containing 0.05 % Tween 20 was used to wash the NC membrane for 10 min×6 times. After the addition of HRP-labeling sheep to rabbit IgG (1 : 10 000), the NC membrane was washed with PBS containing 0.05 % Tween 20 for 10 min×6 times. Color-developing solution was made as follows: 37.5 mg DAB was solved in 150 ml Tris HCl (0.01 mol/L, pH=7.6), then 0.3 CoCl was added for filtration and finally 75 μl H_2O_2 (30 %) was added and mixed completely. The NC membrane was put into the color-developing solution and slightly vibrated at room temperature. The coloration generally lasted 3 h and PBS was used to wash the NC membrane to stop the reaction. Scanning examination was performed by using video scanning densitometer (Japan, CS-930) at 460 nm.

1.2.2 Determination of HSP_{60}, HSP_{70} Level in Lymphocytes The lymphocytes were first splitted. The protein concentrations were made consistent. The determination of HSP_{60}, HSP_{70} levels in lymphocytes were the same as described above.

2 RESULTS

2.1 Plasma HSP_{60}, HSP_{70} Levels

The plasma HSP_{60}, HSP_{70} levels in acute KD, convalescent, and control group were shown in table 1. No significant difference in plasma HSP_{60} level was found among these groups. Plasma HSP_{70} level in acute KD children was significantly higher than that in convalescent group ($P<0.01$).

Table 1 Plasma HSP_{60}, HSP_{70} levels (AS) in KD children ($\bar{x}\pm s$)

HSP	KD groups		Control group ($n=23$)
	Acute ($n=11$)	Convalescent ($n=9$)	
HSP_{60}	3813.8±553.70	4139.19±1334.80	4456.20±1080.11
HSP_{70}	4622.27±787.30*△	3520.99±505.50	4090.90±853.00

*$P<0.01$ as compared with convalescent group, △$P>0.05$ as compared with control group

2.2 HSP_{60}, HSP_{70} Levels in Lymphocytes

The HSP_{60}, HSP_{70} levels in lymphocytes in acute and convalescent KD children, and control group were shown in table 2. The results showed that the HSP_{60}, HSP_{70} levels in lymphocytes in acute KD children were significantly higher than in convalescent group and control group ($P<0.01$ for all).

Table 2 HSP_{60}, HSP_{70} levels (AS) in lymphocytes ($\bar{x}\pm s$)

HSP	KD group		Control ($n=26$)
	Acute ($n=14$)	Convalescent ($n=10$)	
HSP_{60}	9545.10±2596.85*	6347.80±2000.00△	6345.57±1125.15
HSP_{70}	10535.23±2179.30*	7435.08±2107.177△	6679.08±1710.45

*$P<0.01$ as compared with convalescent group and control group,
△$P>0.05$ as compared with control group

2.3 Plasma HSP_{60}, HSP_{70} levels in KD and Febrile Diseases

No significant difference was found in the plasma HSP_{60}, HSP_{70} levels among the KD group, febrile disease group, and control group.

2.4 Comparison of HSP_{60}, HSP_{70} in Lymphocytes between KD Group and Febrile Disease Group

HSP_{60} and HSP_{70} levels in lymphocytes in the acute KD children, febrile disease children and control group were shown in table 3. The results showed that the HSP_{60} and HSP_{70} levels in lymphocytes in acute KD children were significantly higher than in febrile disease children ($P<0.05$) and control group ($P<0.01$).

2.5 Comparison of Plasma HSP_{60} and HSP_{70} Levels between the KD group and Tuberculosis Group

No significant difference in the plasma HSP_{60} and HSP_{70} levels among the children with KD and tuberculosis and control group was found ($P>0.05$).

2.6 Comparison of HSP_{60}, HSP_{70} Levels in Lymphocytes between KD Group and Tuberculosis Group (table 4)

The results showed that the HSP_{60} level in lymphocytes of children with tuberculosis was higher than that in KD children and control group ($P<0.01$). The HSP_{70} level in lymphocytes of children with tuberculosis was significantly higher than that in control group ($P<0.01$).

Table 3 HSP_{60}, HSP_{70}(AS) levels in lymphocytes of KD and febrile disease children ($\bar{x}\pm s$)

HSP	KD group ($n=14$)	Febrile disease group ($n=12$)	Control group ($n=26$)
HSP_{60}	$9545.10\pm2596.85^{*\triangle}$	7295.70 ± 2337.18	6345.57 ± 1125.15
HSP_{70}	$10535.23\pm21779.30^{*\triangle}$	7129.03 ± 2028.68	6679.08 ± 1710.45

$^{*}P<0.01$ as compared with control group, $^{\triangle}P<0.05$ as compared with febrile disease group, $^{\triangle\triangle}P<0.01$ as compared with febrile disease group

Table 4 HSP_{60}, HSP_{70} levels (AS) in lymphocytes of KD children and tuberculosis children ($\bar{x}\pm s$)

HSP	KD group ($n=14$)	Tuberculosis group ($n=10$)	Control group ($n=26$)
HSP_{60}	$9545.10\pm2596.85^{*}$	$18824.20\pm5412.70^{**}$	6345.57 ± 1125.15
HSP_{70}	$10535.23\pm2179.30^{\triangle}$	$10184.27\pm5283.40^{**}$	6679.08 ± 1710.45

$^{*}P<0.01$ as compared with tuberculosis group, $^{**}P<0.01$ as compared with control group, $^{\triangle}P>0.05$ as compared with tuberculosis group

3 DISSCUSION

The recent studies showed that, in the process of microbial infection, autoimmune disease and the cell transformation, the HSPs synthesis was increased appreciably. It is believed that HSP_{60} and HSP_{70} might play important roles in immunological mechanism of KD[2].

3.1 Plasma HSP_{60}, HSP_{70} Levels in KD Children

In this experiment, the plasma HSP_{70} in acute KD children was higher than that in convalescent KD children ($P<0.01$), which was probably due to the fact that HSP_{70} still existed in nucleus after stress period[3], a process called HSP_{70} relocation. Compared with the HSPs levels in lymphocytes, the difference in plasma HSPs levels among the groups is less obvious and this might be related to the distribution and transfer of HSP_{60}, HSP_{70}. Our study showed that the plasma HSP_{60}, HSP_{70} levels were significantly lower than in lymphocytes, indicating that HSP_{60}, HSP_{70} were located intracellularly. This was consistent with the result reported by Bohdan[4].

3.2 The HSP_{60}, HSP_{70} Levels in Lymphocytes in KD Children

The HSP_{60}, HSP_{70} levels in lymphocytes in acute KD children were higher than

those in control group and convalescent KD children, indicating that the products of HSP_{60} mRNA and HSP_{70} expression was increased. Moreover, HSP_{60} and HSP_{70} levels in lymphocytes of KD children was elevated as compared with those in febrile disease children. The results was identical to the results reported by Takeshita[5]. In terms of the characteristics of HSPs and their relation to the pathogenesis of autoimmune diseases, it might be hypothesized that there is a close relationship[6, 7] between KD pathogenesis and HSP_{60}, HSP_{70} families' antigens which can imitate host antigens, stimulate host to produce identical kinds of HSPs, and then trigger autoimmune action. It is believed that HSP_{60}, HSP_{70} are effective immunogens which can activate immunocytes to produce such cytokines as IL-1, IL-6, IFN-γ, TNF-α[8], and cause inflammatory reaction. The increase of the cytokines can promote the transcription and synthesis of endogenous identical family HSPs mRNA to certain extent , and subsequently stimulate the production of the cytokines and start a vicious circle. As a result, the secretion of macrophages is stimulated, and ELAM-1 and ICAM-1 were expressed by vascular endothelial cells. ELAM-1 and ICAM-1 can adhere to LFA-1, which was expressed on the activated immunocytes, so as to cause vasculitis[5].

In this study, the synthesis of HSP_{60} in lymphocytes of KD children was obviously lower than that of tuberculosis children ($P < 0.01$), which may be ascribed to the fact that activated immune system caused the increase of the synthesis of HSP_{60} in lymphocytes of the KD children, but no pathogen existed in the blood[9]. In contrast, the direct action of mycobacteria and activated immune system could induce a higher HSP_{60} level in tuberculosis children than in KD children. No significant difference in HSP_{70} in lymphocytes was found between tuberculosis and KD children, which might be related to the fact that the antigen of mycobacteria mainly belongs to HSP_{60} family, though it includes HSP_{60} and HSP_{70}[10].

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(Received June 17, 1997)