EFFECT OF Hsp70 EXPRESSION ON T-CD4+ LYMPHOCYTE APOPTOSIS IN HIV/AIDS INFECTION

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ABSTRACT

HIV/AIDS until now is still a global health problem including in Indonesia. The effect of Hsp70 expression to T-CD4 lymphocytes apoptosis mechanism in HIV/AIDS infection is still unclear. The objective of this study was to disclose the effect of Hsp70 expression to T-CD4 lymphocytes apoptosis mechanism in HIV/AIDS patients. Methods: Fourteen HIV/AIDS infected patients taken by simple random sampling were enrolled in this research. Fourteen persons having a high risk of HIV infection, but still nonreactive, as revealed in HIV serology test, served as control. All subjects and controls were subjected to three-times examinations for Hsp70, T lymphocytes DNA fragmentation, lymphocytes and CD4 count. The first examination (day 0) was done two hours after the signing of informed consent while the patients still did not know about their HIV infection status. The second examination (day 7) was carried out when the patients were in acute stress condition after HIV/AIDS infection diagnosis was informed. At the third examination (day 31), the patients were in chronic stress condition and showed acceptance of this disease. The results of this research were analysed by multivariate analysis. Results: The first examination revealed that CD4 decreased both in HIV/AIDS infection and non-HIV/AIDS infection group of less than 1000 cells/mm3. The decrease progression in the HIV/AIDS group is faster than that in non HIV/AIDS infection. This study demonstrated that in HIV/AIDS infection group Hsp70 had a higher level than that in non HIV/AIDS infection. The result of the second examination showed that besides the difference of CD4 count in two groups, Hsp70 level increases. It was not only stimulated by biological stress HIV to lymphocytes, but also to other immune cells. Hsp70 level in the second examination was higher than that in the first examination The result of the third examination revealed that Hsp70 level could be readily produced in large amount and it is enhanced by the CD4 expression cells from the two groups. Hsp70 level increased significantly in HIV/AIDS group, more than that in non HIV/AIDS infection at day 31. Hsp70 level in HIV/AIDS group tended to increase from first, second and third examination, i.e., 1.3007 $\pm$ 0.6904 vs 1.5757 $\pm$ 0.8127 vs 1.6907 $\pm$ 0.9175 respectively. This research demonstrated a trend of increasing Hsp70 level from acute to chronic stress in HIV/AIDS infection. In this reseach T lymphocytes DNA fragmentation was found to have less significant difference between HIV/AIDS and non HIV/AIDS group. In conclusion, This study demonstrated that Hsp70 expression can inhibit DNA fragmentation in HIV/ AIDS infection

Keywords: *apoptosis T lymphocytes, HIV/AIDS, Hsp70 expression.*

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INTRODUCTION

The infectious disease HIV/AIDS remains a global health problem, including in Indonesia. Developing problems related with this infectious disease is high incidence and mortality (Hirschel 2003), which results from various factors. One of these factors is related with the apoptosis of T-CD4 lymphocyte that affects HIV progressiveness to AIDS. Hsp70 belongs to chaperone molecular family that has a high contribution to innate immunity as a cell protector from the effect of stress. Excessive Hsp70 expression may induce cellular injury and enhance apoptosis. However, the extent of these two effects of Hsp70 has not been disclosed. The role of Hsp70 on apoptosis should be recognized before determining the extent of the effects. Correlation between Hsp70 expression and T-CD4 lymphocyte apoptosis in defense mechanism has not been clear either. Despite progresses in medical as well as pharmaceutical sciences, and even though preventive actions have been taken, HIV-resulted morbidity rate and mortality rate from AIDS should have been reduced. In fact, those rates remain high, and even tend to increase. The global incidence of infection and death

reveals that 60 million individuals are infected with HIV and 25 million others die from AIDS (DeCock 2008).

HIV infection acts as a stressor for an individual and it is potential to affect Hsp70 expression that affects the apoptosis of T-CD4 lymphocyte and the course of the disease. If the effect of Hsp70 expression on the apoptotic mechanism of T-CD4 lymphocyte has not been immediately disclosed, the management of the patients cannot be performed optimally. A study to disclose the effect of Hsp70 on apoptotic mechanism of T-CD4 lymphocyte in HIV/AIDS patients had been conducted with the hope to optimize management for the patient and to suppress morbidity rate due to HIV and mortality rate from AIDS.

MATERIALS AND METHODS

The study was conducted on the accessible population, i.e., the patients infected with HIV/AIDS, aged 15-65 years at the Intermediary Infectious Disease Treatment Center, Dr Soetomo Hospital, Surabaya. This was an observational study. Subjects were those infected with HIV/AIDS. The purpose of the study was to observe the effect of Hsp70 expression on the apoptosis of T-CD4 lymphocytes in HIV-infected high-risk groups and non-HIV-infected high risk groups as comparison. Each subject from these two groups were followed up for 30 days. This study used panel study design, in which the same subject was subjected to repeated treatment in different time. Both groups were subjected to examination for Hsp70 level, apoptotic T-CD4 lymphocyte, CD4 count and lymphocyte count. In this study, we used comparison from high risk groups but who serologically had not been infected with HIV that might result in the change of Hsp70 level, apoptotic T-CD4 lymphocyte, CD4 count and lymphocyte count.

Since the HIV transmission factor of HIV affects the course of the disease that was expected to influence the result of the study (CD 4 count), the stratification of risk factor types should be performed with the intention to obtain more homogeneous sub-groups (strata). Randomization was carried out in each strata separately, and then selected subjects were recombined within the groups accordingly. Thus, this study employed simple stratified random sampling.

The selection of samples required from HIV-infected patient groups was performed by selection using stratified sampling. To obtain homogeneous sample from HIV-infected high risk group and from high risk group that has not been infected with HIV as comparison, sampling was performed by individual matching in terms of age, sex, nutritional status, type of risks, and the length of contact with high-risk individuals.

Peripheral blood sampling was conducted three times: first examination (baseline data), during which peripheral blood sampling was taken on the first day when the inclusion criteria were met, but the patients had not known of their HIV-infected status. The patient stated no objection in following the procedure of the study and signed informed consent as a subject and medical procedure consent for blood sampling. The second examination was conducted on day 7. It was conducted on day 7 under consideration that the most severe acute stress prevailed on the first seven days after being diagnosed of having HIV infection. The third examination was conducted on day 31 since acute stress generally prevails from two hour to 15 days after obtaining stressor, and followed with chronic stress. Each subject obtained counseling, as well as HIV serological pre-test and post-test.

The inclusion criteria were as follows: high risk group, i.e., heterosexual patient with history of changing partners, homosexual, intravenous narcotics abusers, partners of HIV/AIDS-infected patients who were positively proved as being HIV-infected from serological examination, age 15-65 years, and marriage status, CD4 count more than 200 or more than 200 cells/mm3, without opportunistic infection or malignancy, and stated willingness to participate by signing the informed consent either on the role as subject and medical treatment of blood sampling. The criteria of AIDS used in this study were derived from the results of examination: CD4 less than 200 cells/mm3 without opportunistic infection and malignancy. The exclusion criteria were the presence of condition that could disturb the measurement or interpretation : receiving corticosteroid treatment or other immunosuppressants, the presence of opportunistic infection or malignancy, and the presence of other diseases that could disturb the measurement or interpretation, such as diabetes mellitus, chronic renal disease, and hepatic cirrhosis.

To determine apoptosis, we used ELISA-plus sandwich method. Instrument used in this study was one-step sandwich ELISA, colorimetric that could detect three hours after apoptotic induction. We performed lymphocyte lysis and followed with the immunochemical determination of histone-complexed DNA fragments in microtiter. Test principle was the determination of nucleosome activities using one-step-sandwich immunoassay. The kit consisted of anti-histone antibody (clone H11-4), anti-DNA antibody (clone M-CA-33), and DNA- histone complex. The examination was performed in central Prodia clinical

laboratory, Jakarta. The significance of the kit was that it was able to determine quantitatively the histone-fragment of complex DNA (mono- and olygonucleosome) and cell cytoplasm after apoptotic induction or after being released from necrotic cells.

The result of Hsp70 concentration was determined from the mean absorption of each standard and samples, and the absorption size from standard 0 ng/ml Hsp70 was determined. The mean substrate obtained wast standard (0 ng/ml) Hsp70 from values obtained from the first step (standard and sample). We determined the scale of recombinant Hsp70 standard level (ng/ml) in X axis, and the absorption was measured according to Hsp70 standard in Y axis. The determination of sample concentration was done from standard curve and multiplication with the dilution of sample Hsp70 concentration. For example, if the sample was diluted 1 : 25 before measurement, the subsequent rate from standard curve should be multiplied with 25 at Hsp70 level.

RESULTS

Result of study from HIV-infected group and non-HIV-infected group on examination day 1 was as follows: lymphocye count 1.6271 ± 0.4349 vs 1.7171 ± 0.4540 ; CD4 count 250.29 ± 230.15 vs 598.50 ± 370.70 ; Hsp70 level 1.3007 ± 0,6904 vs 0.8214 ± 0.3157; and cell death 0.29307 ± 0.43161 vs 0.33371 ± 0.24749.

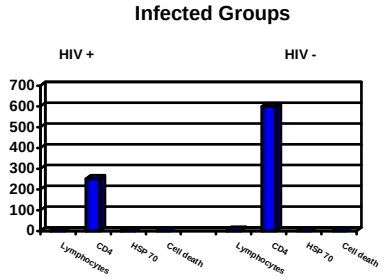

Figure 1. Hsp70 level, apoptotic T-CD4 lymphocyte, CD4 count, lymphocyte count on examination day 1.

Result of study from HIV-infected group and non-HIV-infected group on examination day 7 was as follows: lymphocyte count 1.7271 ± 0.3202 vs 1.7507 ± 0.2237 ; CD4 count 227.71 ± 200.58 vs 677.86 ± 488.61 ; Hsp70 count 1.5757 ± 0.8127 vs 0.9579 ± 0.3969; and cell death 0.34036 ± 0.52735 vs 0.42871 ± 0.47416.

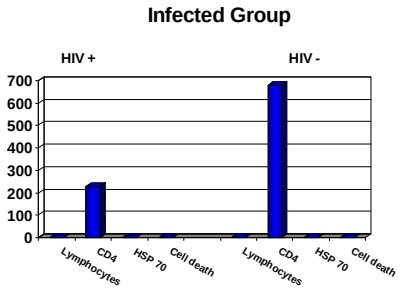

Figure 2. Hsp70 level, apoptotic T-CD4 lymphocyte, CD4 count, lymphocyte count on examination day 7.

Result of study from HIV-infected group and non-HIV-infected group on examination day 31 was as follows: lymphocyte count 1.6029 ± 0.3891 vs 1.7586 ± 0.2819; CD4 count 305.14 ± 265.00 vs 598.79 ± 373.57; Hsp70 level 1.6907 ± 0.9175 vs 0.8464 ± 0.3608; and cell death 0.30829 ± 0.40400 vs 0.72357 ± 0.68682.

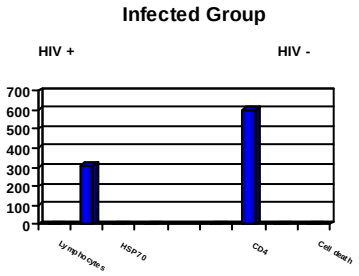

Figure 3. Hsp70 level, apoptotic T-CD4 lymphocyte, CD4 count, lymphocyte count on examination day 7.

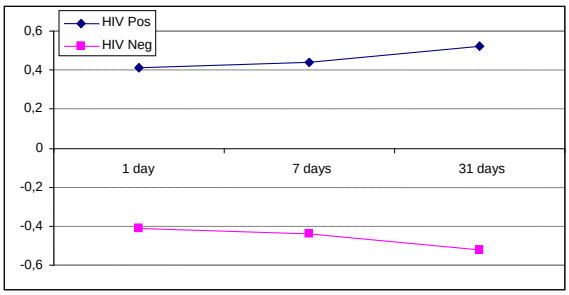

Figure 4. The change of Hsp70 on observation days 1, 7, and 31.

Positive HIV group had Hsp70 level increase in examination day 7 and 31, compared to the examination on the first day. Negative HIV group showed decrease on examination day 7 and decreased further on day 31. It could therefore be inferred that Hsp70 contribution to HIV-infected group was more predominant in inhibiting apoptosis progress.

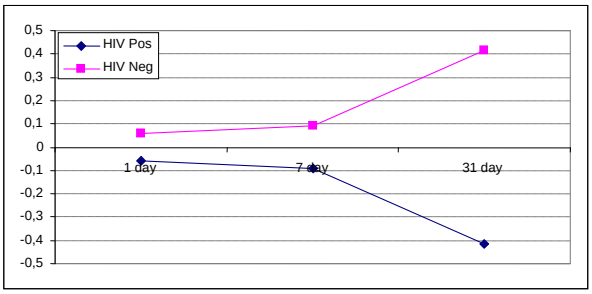

Figure 5. The change of total cell death on observation days 1, 7, and 31.

Total cell death in positive HIV group showed decrease on examination day 7, and sharply decreased on examination day 31. Negative HIV group showed increased total cell death on examination day 7 and tended to continuously increase sharply on examination day 31.

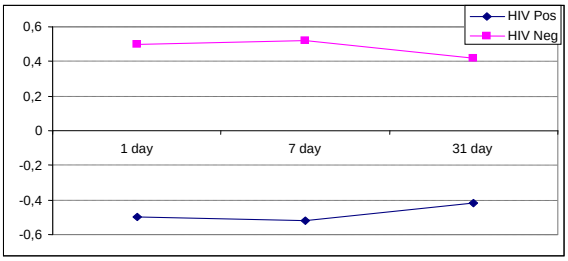

Figure 6. The change of CD4 count on observation days 1, 7, and 31

In positive HIV group, examination on day 1 and 7 showed total CD4 reduction, but the value on examination day 7 was slightly lower than that of day 1, and tended to increase up to the examination day 31. Negative HIV group showed slight increase and tended to decrease until the examination day 31.

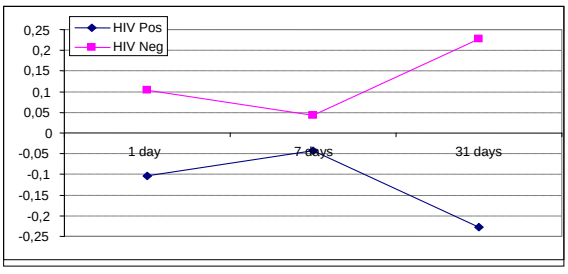

Figure 7. The change of CD4 count on observation days 1, 7, and 31.

Positive HIV group showed sharp reduction of lymphocyte count on day 31. Negative HIV group revealed transient reduction on day 7 and increased on day 31.

DISCUSSION

Increasing Hsp70 expression enhances resistance against apoptosis (Mosser 2000). Stress may induce apoptosis starting by the release of cytochrome C from mitochondrial intermembrane space. Cytochrome C binding with ATP, which subsequently induced apoptotic protease–activating factor-1 (Apaf-1) within cytoplasm, causes Apaf-1 to expose N-terminal procaspase 9. Along with apoptosom, procaspase 9 activates caspase 9 and affect the activation of caspase 3, 6 and 7, that results in cell death. Increased Hsp70 expression may blockade the death process through the blockade on poly (ADP-ribose) polymerase caspase 3. The protection by Hsp70 may affect the reduction of cytochrome C release by inhibiting cytochrome c-dATP.

Various factors determine the course of HIV infection. One of the important factors is enhanced attenuation of immune status, characterized with the reduction of T-CD4 lymphocyte count. In this study, predominant stress protein in the first examination is marked by the increase of Head shock protein70 (Hsp70) level. The predomination of Hsp70 level was found in the first examination when the individual had not known his serological HIV status. Increased Hsp70 level may occur due to the effect of stress internal environment triggered by HIV biological stressor. HIV biological stressor affects the expression of CD40 receptor on the outer surface of the lymphocyte. The CD40 receptor was intended to Hsp70 level (Lehner 2005). Therefore, it is natural when HIV inductor triggers the increase of Hsp70 level in HIV-infected group as demonstrated by the results of this study.

Stress cell may ignite its own destruction through apoptosis (Mosser 2000). In facing stress, cell produces response for adaptation by increasing tolerance and staying normal. Hsp70 expression may enhance cell protection against the threat of apoptosis. Initially, excessive Chaperonins expression is aimed to prevent the formation of protein aggregate and prevent to occurrence of T-CD4 lymphocyte apoptosis in HIV-infected patients (Kobayashi 2000). Hsp70 is known to have either upstream or downstream role in caspase activitve, and it has protective influence, dependently or independently, on the blockade of JNK activities. Hsp70 blockades procaspase 3 and 9, preventing the formation of active caspase 3 and 9. Hsp70 also inhibits the release of cytochrome c in mitochondria (Mosser 2000).

The reduction of CD4 confirms the result of this study. Both HIV-infected group and serologically non-HIV-infected showed a predisposition of CD4 reduction to less than 1,000 CD4/mm3. The presence of Hsp70 was

induced by the effect of HIV biological stressor that had intervened T-lymphocyte and incrased the expression of CD40. The result of this study indicated that the increase of Hsp70 level was more predominant on examination day 1. This was possible because Hsp70 belongs to small protein (kDa) needed by HIV as ligand. HIV itself requires the role of CD40 receptor in order to bind Hsp70 (Hsp70 as CD40 ligand).

The second examination revealed the increase of Hsp70 level. In the second examination (day 7) the mean Hsp70 level was higher than that in the first examination. High Hsp70 level on examination day 7 can be explained if in the first examination the Hsp70 was induced more by HIV biological stressor that had successfully intervened the lymphocyte through the activation of CD40 receptor (Gill, 2004). The increase of Hsp70 level occurred in order to prevent the apoptosis of T-lymphocyte through the Hsp70 cytoprotective characteristic at the early phase. The increase of Hsp70 in this study, which resulted from the effect of HIV biological stressor whose effect was increasing along the course of time. The strength of the effect is related with HIV replication that was continuously undergoing with mean speed of 10^9 - 10^{11} virus particle daily, resulting in HIV density (viral load) within the circulation (Drew 2001; Hirschel 2003). In addition, T-lymphocyte and the effect of CD-4 expressing cells were also simultaneously increasing. HIV that followed the systemic circulation could affect various cells that had CD4 protein on their surface, such as astrocyte, microglia, monocyte-macrophage, dendrites, and Langerhan's. Those various cells were activated to produce Hsp70. Epithelium on the surface of digestive tract was also activated by the direct effect from HIV gp41 (Pavlakis, 1997). The epithelium is also potential to express Hsp70 by the presence of CD40 receptor, which is the receptor of Hsp70 (Bhattacharyya 1999; Stuart 1998).

The examination on day 3 also revealed the increase of anti-Hsp70 level, indicating that immune system cells in subject groups in this study remained providing responses against Hsp70. Its response was decreaseng in the course of time, particularly in group infected with HIV. Hsp70 is a molecular chaperone activated by various conditions, including heat stress condition. Hsp70 will be coordinated with chaperone and other cochaperones and interacted intra-molecularly between N-terminals and inter-molecularly aas well C-terminal domain. The molecular activity of chaperone heat shock protein 70-kDa (Hsp70) was in its function, prevent aggregation, and the regulation of important cellular protein stability, including steroind hormone receptor, protein kinase, cell cycle regulation and programmed cell deat, including termasuk calmodulin and Raf1 (Bhutani 2002; Nollen 2002). Such condition demonstrates the presence of various peptide binding sites to Hsp70. If a cell is faced to stressful condition, Hsp, including Hsp70, has an important role to protect cells from stress, meaning that is has cytoprotective effect (Beck 2000; Bhattacharyya 1999; Nollen 2002). Hsp assists protein folding process through a step that requires ATP energy (Artika 2004). The increase of Hsp70 expression may increase more the resistance against apoptosis, as confirmed in this study. The increase of Hsp70 expression is able to blockade such death process through the blocking of poly-(ADP-ribose) polymerase caspase 3. Protection by Hsp70 may affect the reduction of cytochrome c release by inhibiting c-d ATP cytochrome (Mosser 2000).

In relations with CD4 reduction, both HIV-infected group and the group that serologically had not been infected with HIV demonstrated predisposing decline of CD4 to less than 1,000 CD4/mm3. The rate of CD4 reduction in HIV-infected group is more dramatic than that in group not yet infected with HIV. In HIV-infected group the reduction of CD4 took place more progressively since T-lymphocyte is the primary target of HIV. T-lymphocyte, in addition to the presence of CD4 receptor on its surface, is also equipped with two co-receptors, CCR5 and CXCR4 that enhance binding with gp120 on the outer surface of HIV. In non-HIV infected group, CD4 reduction may still be possibly due to HIV intervention, but the antibody had not been detectable laboratorically.

In this study, the examination of DNA fragmentation to identify the death of T-lymphocyte did not reveal difference between groups that were infected and that serologically not yet infected. The result of the first examination (0.29307 ± 0.43161 vs 0.33371 ± 0.24749), second examination (0.34036 ± 0.52735 vs 0.42871 ± 0.42971), and third examination (0.30829 ± 0.40400 vs 0.72357 ± 0.68682) revealed no significant difference between both groups. Similarly, from the first to the third examination there was no predisposing increase of progressive cell death. In group that serologically has not been infected with HIV, the apoptosis of T-lymphocyte was lower than that in HIV-infected group. Such condition can be explained in this study, that there was an increase of Hsp70 as cytoprotector in HIV-infected group during the longitudinal study for 30 days. On the other words, it indicated that the body was still capable to inhibit the progress of apoptosis in T-lymphocyte in a period of 30 days. To ascertain the death of T-lymphocyte through apoptosis, a longer period is required, along with the increasing reduction of CD4 count in chronic phase with a reduction rate of 70 cells/mm3 annually (Hirschel, 2003). T-CD4 lymphocyte apoptosis is only one of cell death sequence

(Badley 2000). It was strongly suspected that disease progressiveness and death was through the apoptosis since there was no clinical evidence, that was the biological parameter through necrosis that should have been accompanied with accumulation of inflammatory cells. Excessive Hsp70 expression induced cell injury due to exhaustion. Unexpected death, which often occurs in HIV/AIDS patients, based on biological parameter from this study, was strongly suspected as being resulted from diffuse pathological apoptosis, which was enhanced after the cells failed to maintain homeostatic condition. The result of this study ascertained that enhanced diffuse pathological apoptosis did not occur before day 31 after the diagnosis of HIV/AIDS-infected was informed.

CONCLUSION

The increase of Hsp70 level is along with the decrease of total cell death and related with early acceptance in chronic stress. Decreasing T-CD4 lymphocyte can be inhibited by the role of Hsp70 cytoprotective role. In certain condition, it becomes Hsp70 stimulator that has an important role in cytoprotective mechanism against stressful microenvironment. As a chaperone molecule, Hsp70 prevents protein aggregation, regulates protein folding rhythm, and maintain functional three-dimensional protein structure. As a ligand, Hsp70 may have protective effect on the apoptosis of T-CD4 lymphocyte in HIV infection.

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